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Development of new anti-bacterial agents

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Development of new anti-bacterial agents

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M.Sc. (Medicinal Chemistry)

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Declaration

I, **Muni Kumar Mahadari**, declare that this thesis is submitted in fulfillment of the requirements for the conferral of the degree Doctor of Philosophy, from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

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Muni Kumar Mahadari

March 4th, 2021

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Abbreviations

°C	degrees Celsius
R_f	retardation factor
%	percentage
δ	chemical shift in ppm
ν_{max}	wavenumber of maximum absorption peak in cm^{-1} (IR data)
μg	microgram
+ve	positive (electric charge)
–ve	negative (electric charge)
1-D	one-dimensional
2-D	two dimensional
app.	apparent (NMR)
AcOH	aceticacid
Arg	arginine
Boc	*t*-butyloxycarbonyl
brs	broad singlet
calcd	calculated
CC_{50}	concentration at 50% cytoxicity
CDC	Centres for Disease Control
CDI	*Clostridium difficile* infection
ClogP	calculated partition coefficient (Log P)
cm	centimeter(s)
conc.	concentrated
CuAAC	Cu-catalysed azide alkyne cycloaddition
d	doublet (NMR)
dd	doublet of doublet (NMR)
dt	doublet of triplet (NMR)

DBF	dibenzofulvene
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-*d*6	deuterated dimethyl sulfoxide
DNA	deoxyribonucleic acid
EDCI	1-[3-(dimethylamino)propyl]-1-ethylcarbodiimide hydrochloride
eq	equivalence
ESI	electrospray ionization
ex	excitation wavelength (fluorescence)
FDA	Food and Drug Administration
Fmoc	fluorenylmethyloxycarbonyl
g	gram(s)
gCOSY	gradient correlation spectroscopy
gHMBC	gradient heteronuclear multiple bond correlation
gHSQC	gradient heteronuclear single quantum correlation
GI	gastrointestinal
h	hour(s)
HOBt	1-hydroxy-1*H*-benzotriazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry (or spectra)
Hz	hertz
IR	infrared (spectroscopy)
J	coupling constant (NMR data)
L	litre(s)
LRMS	low resolution mass spectrometry (or spectra)
Lys	lysine
M	molarity (units of concentration = mol/litre)

m	multiplet (NMR)
m/*z*	mass to charge ratio (MS data)
mbar	millibar (unit of pressure)
MBC	minimum bactericidal concentration
mg	milligram(s)
MIC	minimum inhibitory concentration
min	minutes
mL	millilitre(s)
mmol	millimole(s)
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	mass spectrometry (or mass spectrum)
nm	nanometers
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
OD600	optical density observed at 600 nm
*p*TSA.H_2O	*p*-toluenesulfonic acid monihydrate
Pbf	pentamethyldihydrobenzofuran-sulfonyl
ppm	parts per million
Cp*RuCl(PPh_3)$_2$	pentamethylcyclopentadienylbis(triphenylphosphine) ruthenium(II) chloride
q	quartet (NMR)
RNA	ribonucleic acid
rt	room temperature
SiO_2	silica
s	singlet (NMR)
SAR	structure-activity relationship
THF	tetrahydrofuran
TMS	tetramethylsilane
TEA	triethylamine

t	triplet (NMR)
td	triplet of doublet (NMR)
TFA	trifluoroacetic acid
TLC	thin layer chromatography
USD	United States dollars
UV	ultraviolet
v/v	volume-to-volume ratio
VRE	vancomycin-resistant *Enterococcus faecium*
VRSA	vancomycin-resistant *Staphylococcus aureus*
VT-NMR	variable temperature NMR
w/v	weight-to-volume ratio
w/w	weight-to-weight ratio
zTOCSY	z-quantum total correlation spectroscopy

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Abstract

Clostridium difficile is a spore-forming, anaerobic Gram-positive bacteria that results in infection in the gastrointestinal (GI) tact. *C. difficile* has been known as the costliest bacteria in terms of human mortality and financial burden. Furthermore, the current treatments that exist for *C. difficile* infection (CDI) are inefficient and expensive and often result in disease reoccurrence due to the release of spores. Therefore, it is an important medical requirement to develop novel antibacterial compounds to effectively treat CDI. As such this PhD project has investigated the modification of the binaphthyl hydrophobic region of the lead compound-1 **(AVX-13616)** with alkyl and benzyl substituted phenyltriazole and naphthalenetriazole peptidomimetics towards the discovery of new CDI inhibitors.

An effective and modular synthetic route was developed to prepare twenty-nine novel mono- and di-cationic peptide derivatives which incorporated a phenyltriazole or naphthalenetriazole group with the aim to achieve better water solubility over previously prepared analogues by our group which were difficult to deliver in mouse model experiments because of their poor water solubilities. Chapter 2 describes the successful synthesis of these analogues. In some cases, the attempted synthesis of the 1,5-triazole moiety using the Ru-catalysed click reaction were unsuccessful when sterically hindered azide and alkyne substrates were employed. We found, however, that the magnesium promoted click reaction conditions reported by Sharpless *et al.* were useful for the synthesis of these sterically hindered 1,5-triazoles.

The difficulties encountered in preparing sterically hindered 1,5-triazoles initiated a general study into this synthetic problem. The results of this study are reported in Chapter 4.

The new analogues prepared here were tested against a wide range of bacterial strains and two fungal strains by collaborators at three independent laboratories. These results are reported in Chapter 3. The twenty-nine peptide derivatives had variable minimum inhibitory concentration (MIC) values from the antimicrobial screening. All of the compounds displayed MIC values in the range of 2 – 128 μg/mL against both Gram-positive and Gram-negative bacterial pathogens. The naphthyl-triazole dicationic compounds **42** and **43** showed the best broad-spectrum antibacterial activities, displaying MIC values of 8 μg/mL against *S. aureus*, 8 μg/mL against MRSA, 8 μg/mL against *E. faecalis* and 8 – 16 μg/mL against *S. pneumoniae*. The compounds, **29 – 30**, **33** and **43 – 45**, exhibited weak antifungal activity (MIC = 8 **–** 32 μg/mL) against *C. albicans* and *C. neoformans*. The compounds **41** and **42**, however showed MIC values of 4 μg/mL against *C. neoformans*.

Compounds **41** and **43** (naphthalenetriazole derivatives) exhibited similar antibacterial activity against *C. difficile* (MIC = 8 μg/mL); these molecules were also structurally similar except for the nature of their hydrophobic termini (cyclohexyl and benzyl, respectively). A cytotoxicity analysis revealed that these compounds had relatively low cytotoxicity (CC_{50} = 32 μg/mL). Overall compound **41** showed similar antibacterial activity against *C. difficile* (MIC = 8 μg/mL) to the lead compound-1 (MIC

= 8 μg/mL), better water solubility (5 times better than lead compound-1) and low cytotoxicity (CC50 = 32 μg/mL); these properties favored compound **41** for further *in vivo* testing in a CDI mouse model.

Compound **41** was synthesized on a larger scale (≈ 300 mg) for further studies in an *in vivo* CDI mouse model to identify its potential as an *in vivo* CDI inhibitor. At 24 hours post-infection, compound **41** appeared to be protecting the mice from disease as the mice lost the least amount of weight and had mild diarrhoea. Furthermore, compound **41** displayed some promising activity, despite not completely protecting the mice over the second day period of this trial and the mice had to be culled after 42 h due to weight loss from the disease.

In summary, a total of 29 novel phenyltriazole and naphthyltriazole peptide derivatives were synthesized *via* a modular synthetic route and tested against various Gram-positive and Gram-negative bacteria. Compound **41** showed better antibacterial activity against *C. difficile*, good water solubility and low cytotoxicity; these properties favored the compound for further *in vivo* testing in a CDI mouse model. Compound **41** displayed some promising activity at 24 h, despite not completely protecting the mice in the murine model of CDI study and the mice had to be culled after 42 h.

1.0 – Introduction

1.1 – Resistance to Antibacterial agents

Antibacterial resistance to known antibiotics is, and will continue to be, a severe threat to the current human healthcare services and overall security.[1-6] Inflammation disease is the second biggest medical threat globally (17 million deaths/year) and bacterial infections from antibiotic resistant is presently viewed as a "rising universal disease" by the World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC).[6-7] The CDC evaluates that antibiotic resistance is responsible for 23,000 deaths and more than two million inflammation diseases every year in the USA.[2] The WHO has begun an "Universal Action Plan on Antimicrobial Resistance" around the world to control the severity of drug resistance. Both the WHO and CDC have released a list of drug resistance microbes that are presently thought to be a critical threat to current human healthcare services and overall security.[8] Bacterial strains have developed resistance to the most common antibacterial agents and the regularity of hypervirulent strains just potentiates the problem.[9-10] Therefore, the requirement for novel antibacterial agents with new mechanisms of action is urgently needed.

Bacterial resistance arises in three ways: 1. natural resistance in certain types of bacteria, 2. genetic mutation and, 3. by one bacterium acquiring resistance from another *via* horizontal gene transfer.[9] All classes of microbes can develop resistance, including fungi and viruses. Bacterial resistance to antibiotics has developed quickly since the introduction of penicillin during the 1930s, and 85% of clinically isolated Staphylococci became penicillin resistant by the end of the 1950s.[9]

Vancomycin was quickly permitted for use as an antibacterial agent because its effective treatment against penicillin resistant bacteria was needed urgently.[11] Methicillin, the first semi-synthetic penicillin, was developed by Beecham in 1959 and was rapidly introduced as an antimicrobial agent because of its efficacy against penicillin resistant bacteria.[11] The consequent rise of methicillin-resistant *Staphylococcus aureus* (MRSA) and its developed resistance to most other common antibacterial agents led to vancomycin becoming “the best medicine" for the treatment of MRSA. This led to an increase in vancomycin usage to treat MRSA and also to treat the rising cases of *Clostridium difficile* infection (CDI) during the 1970’s. The massive consumption of oral vancomycin for the treatment of CDI caused the rise of vancomycin resistant *enterococci* (VRE) in 1986. Vancomycin resistant *S. aureus* (VRSA) additionally developed *via* parallel gene transmission from VRE in a patient in 2002, who had been occasionally treated with vancomycin.[11] Some microbes have developed resistance against many antibacterial agents and these multi-drug resistant (MDR) pathogens are a major concern for the current healthcare system. Informally the resistant bacterial isolates are called "superbugs" and are currently a serious concern and therefore a strong motivation for the invention of new antibacterial agents.[12-13]

Recently, vancomycin has been supplanted to treat VRSA, MRSA and all Gram-positive microbial diseases by tigecycline, linezolid, daptomycin and quinupristin/dalfopristin.[10-11] Both linezolid and daptomycin have been used generally to treat Gram-positive diseases; in this way, resistance of these two antibacterial agents has been documented recently.[14] Gram-negative microorganisms are normally less

impervious to antibacterial agents because of their impermeable external membrane and their efflux pumps (for eliminating undesired chemicals out of the cell).[13] Multi-drug resistance in Gram-negative microorganisms is rapidly increasing, so there is an urgent need for the development of novel antibacterial agents with better efficacy compared to present antibiotics to treat such related inflammations.[13] Thus, antibacterial drug discovery has shifted its focus on the development of novel antibacterial agents against MDR Gram-negative microbial inflammations.[13] The **ESKAPE** microorganisms (i.e. ***E****nterococcus faecium*, ***S****taphylococcus aureus*, ***K****lebsiella pneumoniae*, ***A****cinetobacter baumannii*, ***P****seudomonas aeruginosa* and ***E****nterobacter* spp.) are six anti-microbial resistant bacterial strains that have been perceived by the Infectious Diseases Society of America (IDSA) as the key organisms behind most nosocomial inflammations.[5] These microorganisms have been classified based on their harmfulness, steadiness, tendency for obstacle and pathogenicity.[5] Remarkably, *P. aeruginosa* and *A. baumannii* showed an in-built ability to oppose antibacterial agents, probably due to their capability of surviving in hazardous environments. The lack of sustainable choices of medication for Gram-negative microbial infections has even led to the reintroduction of polymyxin B and polymyxin E (colistin), despite known toxicity issues – polymyxins are relatively large cyclic cationic peptides with potent antimicrobial activity.[13]

Regrettably, microbial resistance is unavoidable, and each bacterium will eventually develop resistance to any antibacterial agent. There is an increased chance for the development of a resistant bacterial strain by increased exposure to antibiotics. In this way, the increased use of antibiotics by the agriculture industry for animal growth – (>

50% of antibiotics consumption) and the abuse and overuse by modern medicine has worsen the problem of resistance by accelerating the process.[15] In 2015, a five year plan was announced by the US White House on abuse of antibacterial agents in the agriculture industry where this is a major issue.[7] Furthermore a program called *"National Action Plan for Combating Antibiotic-Resistant Bacteria"* was announced by the US White House – the objective of this program was financial support to novel anti-microbial drug discovery research for the treatment of MDR bacterial infections.[7] The resistance of pathogenic bacteria toward antibacterial agents has become a serious issue, so the WHO recently cautioned of a 'post-antibiotic time'; where regular diseases and minor injuries will demonstrate as deadly because of the absence of reasonable anti-microbial therapies.[16] Thus, novel antibacterial agents with distinctive mechanism of actions are desperately required to tackle the rising problem of bacterial resistance.

1.2 – Clostridium difficile infection

C. difficile is a spore-forming, anaerobic Gram-positive microbe that results in moderate to severe illness in the gastrointestinal (GI) tact. This bacterium produces toxins and resilient spores (Figure 1.1).

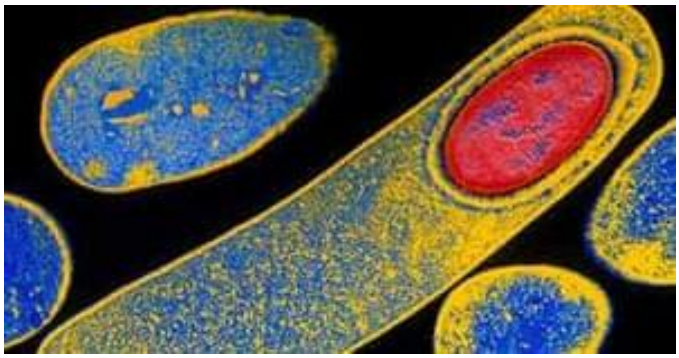


Figure 1.1 – *C. difficile* forming an endospore (red).[19]
(Electron micrograph pic)

C. difficile infection (CDI) happens when the common GI microbiota is compromised; this licenses *C. difficile* to grow well in the GI tract. Most standard oral antibiotics, as well as penicillins, can target the healthy GI microflora – this undesirable effect gives *C. difficile* up to a 20% chance to survive in the gut.[17] *C. difficile* produces vigorous endospores that are spread by the fecal-oral course; these spores are impenetrable to most anti-infectives, hand sanitizers and heat. The hospital environment allows ideal conditions for *C. difficile* spores, which can live for a significantly long time and helps the spread of *C. difficile* spores via nosocomial contamination; also known as hospital acquired contamination which usually occurs via healthcare workers, patients and hospital equipment. The other major threat of CDI is earlier antibiotic treatment.[18] Broad range anti-microbial treatment commonly destroys the GI microflora while treating *C. difficile* infection. Most of the broad-spectrum antibacterial agents such as fluoroquinolones, ampicillin/amoxicillin, clindamycin and cephalosporins eradicate the commensal GI microbiota while treating CDI.[17, 20]

Patients having a *C. difficile* contaminated GI system endure indications such as diarrhoea, abdominal cramping, abdominal pain and pseudomembranous colitis (PMC – a serious gastric illness). CDI-related PMC can be so serious as to warrant colectomy (i.e. resection of the colon) for which there is a ~55% death rate for patients that proceed to medical surgery.[21] Furthermore, poisonous megacolon can arise because of CDI, wherein the patient encounters serious colonic irritation and stomach distension.[20] CDI shows an overall demise rate up to 8%[18] and re-occurrence of disease occurs in up to 20% of cases treated with first-line drugs (vancomycin or metronidazole).[22] Once the spores have reached to the GI, the accessible bile salts initiate germination of the spores to convey the vegetative *C. difficile* cells. The illness obtained by CDI basically creates two destructive elements (Toxin A and Toxin B) that are released by the vegetative *C. difficile* cells; these toxins develop colonic disease and damage epithelial tissue which causes loss of liquid (diarrhoea). A third toxin (known as CDT or matched toxin) is found in significant levels in hypervirulent *C. difficile* strains; the role of this poison in disease has not yet been elucidated.[20]

As of late, both the earnestness and recurrence of CDI cases have increased due to epidemics of hypervirulent *C. difficile* strains, for example ribotype 027 – also known as strain B1/NAP1/027.[17] The hypervirulent ribotype 027 produces larger quantities of toxins than common *C. difficile* strains – these poisons are responsible for the infirmity side effects of diarrhoea and PMC.[18] Thus, ribotype 027 shows higher death rates than normal *C. difficile* isolates.[17]

The inescapability of *C. difficile* contamination invokes an enormous monetary burden on the current therapeutic services administrations (>$1 billion every year in the USA);[17, 23] another assessment put CDI-related human medicinal services administration costs at $4.8 billion in 2008.[18] The CDC gave a report in 2013 entitled 'Resistance Threats to Antibiotics' in which *C. difficile* was noted as the prime microbial threat to humans and the toughest test for the social insurance system.[3] A continuous update by the CDC revealed that CDI is responsible for approximately 500,000 diseases and 15,000 deaths consistently in the USA – the largest increase in any of the bacterial threats recorded by the CDC.[23] Due to the elevated levels of illness, expenditure, recurrence, deaths and the absence of satisfactory therapies for CDI, there is considerable motivation to pursue innovative antibacterial agent that successfully prevent CDI.

Antibacterial selectivity for *C. difficile* is an important factor for a relatively efficient CDI antibacterial agent. The elimination of all GI microbiome by broad-spectrum antimicrobial agents results in an ideal environment for *C. difficile* spores to develop; thus, the maintenance or restoration of commensal GI microflora is necessary for stopping CDI recurrence.[17-18, 20] This explains why CDI is so difficult to treat with conventional antimicrobial agents and why CDI reoccurs in 15-30% of patients who have been treated with vancomycin.[17] Fecal transplantation has become a feasible strategy for treating cases of severe CDI, as it replaces the commensal GI microflora physically.[17-18, 20] The typical antibacterial agents (i.e. metronidazole and vancomycin) suffer from efficacy concerns, especially CDI recurrence due to their lack of selectivity for *C. difficile* and their lack of ability to prevent sporulation.[17-18] It has been found that CDI recurrence is associated with

the lack of ability to develop a specific antibody response to the toxins released by *C. difficile*; healthy adults naturally develop antibodies against toxins A and B.[17] Non-chemotherapeutic treatments (i.e. medical surgery) are available, but they are only utilized when traditional drugs have been unsuccessful. Surgical treatments are permanent – most involve subtotal colectomy in an effort to substantially reduce the infection and diseased bowel.[18] Existing *C. difficile* medications fail to treat CDI with no recurrence. Thus, there is a demand for more efficacious antibacterial agents for CDI treatment. A well-tolerated and reliable drug that can selectively target *C. difficile* (and its spores), without effecting the GI microbiome intact, would be vital in the treatment of severe and recurrent CDI.

1.3 – Available medicines for *C. difficile* infection (CDI)

There are currently only three medicines used to treat CDI. They are vancomycin, metronidazole and fidaxomicin.[20, 22, 24] Only vancomycin and fidaxomicin are FDA approved for treatment of CDI. Vancomycin was the only FDA approved treatment for CDI until 2011 when fidaxomicin was also approved. Metronidazole is widely used to treat CDI in mild to moderate and recurrent cases despite not having FDA approval.

1.3.1 – Metronidazole

Metronidazole (Figure 1.2) is an imidazole derivative that has been utilized initially as an antiprotozoal medicine to treat *Trichomonas vaginalis* and giardiasis, eventually found to be effective against both Gram-positive and Gram-negative pathogens for over 45 years. It can be utilized either alone or with different antibacterial agents to

treat inflammatory diseases. It is a possible medicine for mild CDI if vancomycin or fidaxomicin is inaccessible.[27-28]

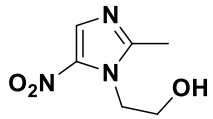


Figure 1.2 – Metronidazole

Metronidazole is marketed under the brand name Flagyl, Filmet and Metro. During the 30 years of utilization, metronidazole was eminent because a low resistance profile was observed.[28] Metronidazole can be administered orally, intravenously and topically and shows ideal pharmacokinetics for a systemic anti-infective. Metronidazole is an inexpensive medicine that has a straightforward structure that permits efficient and cost-effective bulk production (~$0.86 USD/dose).[25]

Metronidazole shows potency through a free radical mechanism where the nitro group is reduced *in vivo* to a reactive 'nitro radical anion' which is unstable and readily decomposes to give an imidazole radical and a nitrite ion.[20, 28] The imidazole radical and nitrite ion cause oxidative damage to DNA strands, resulting in apoptosis. Metronidazole has supplanted vancomycin to treat milder illness of CDI as it is less expensive and has a comparative efficacy profile.[25] Most orally taken metronidazole is absorbed systemically which is not suitable for *C. difficile* treatment.[27-28] Low oral bioavailability is fundamental for treatment of CDI as the drug must stay and remain in the gut and not be absorbed systemically.

1.3.2 – Vancomycin

Vancomycin (Figure 1.3) is a glycopeptide antibacterial agent that is utilized for the treatment of various bacterial inflammations. This compound was developed by the Eli Lilly pharmaceutical organization in 1959. Vancomycin was isolated from a soil sample from the rainforests of Borneo from the microorganism *Amycolatopsis orientalis.*[9] Fermentation of *A. orientalis* efficiently yields vancomycin on an industrial scale. This production technique is financially more viable than total synthesis due to its complex chemical structure.[11] Vancomycin costs ~$31 USD per dose; which is relatively expensive compared to the synthetically feasible small-molecule medications (e.g. metronidazole).[9, 25]

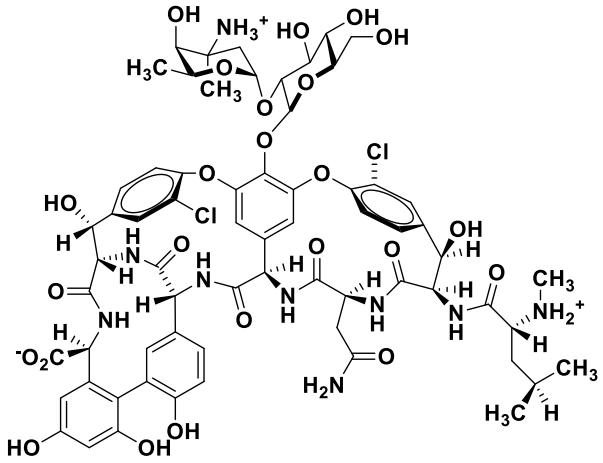


Figure 1.3 – Vancomycin (glycopeptide antibiotic)

The glycopeptides represent a complex class of molecular structures; the main tricyclic structure comprises peptide, ether and biphenyl moieties with a disaccharide attached to one of the phenol rings. Vancomycin is known to work by obstructing the development of the bacterial cell wall. The binding of vancomycin to the terminal *D*-alanine amino acid of Gram-positive microorganisms forestalls the transglycosylase

enzyme from cross-connecting the peptide as a component of the bacterial cell wall.[9] Vancomycin displays antibacterial activity (MIC) ≤ 0.50 μg/mL against *C. difficile* and 207 *C. difficile* strains.[26] Vancomycin displays numerous traits that make it perfect for extreme CDI treatment such as a low oral bioavailability and a potent MIC against *C. difficile*. But unfortunately, it has a few disadvantages i.e. lack of selectivity for *C. difficile*, recurrence of CDI following treatment, cost and production.[11, 25] In particular, vancomycin plays a significant role in treating MRSA and other MDR microbes and thus it will never be given full permit for the treatment of mild or moderate CDI.

1.3.3 – Rifaximin

Rifaximin (Figure 1.4) is from the rifamycin group of semi-synthetic antibiotic. This drug is amazingly highly potent against *C. difficile* and furthermore displays broad-spectrum activity.

Rifaximin showed an antibacterial activity (MIC) of ≤ 0.015 μg/mL against 110 pathogenic *C. difficile* strains while maintaining low solubility in water.[25, 31] After oral dosing, the intestinal concentration of this drug remains high with 97% of the dose recovered as unaltered rifaximin in the faeces. The drug maintains systemic bioavailability under 0.4% – which is ideal for CDI treatment.[32]

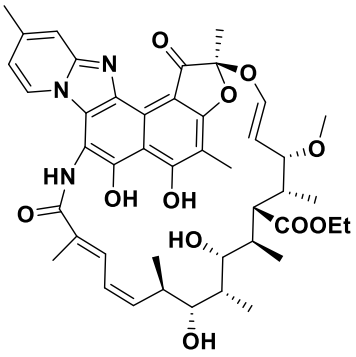


Figure 1.4 – Rifaximin

Ongoing investigations have shown that rifaximin can be used for resistant CDI treatment – as a "salvage therapy" where several classic drugs have failed to do.[25, 33] These examinations indicated that the drug is well-tolerated by most patients and that it has had some success in cases such as resilient CDI treatment, including a few strains containing the NAP1/BI/027 ribotype.[33] *C. difficile* resistance against rifamycin is growing as an issue with around 2% of present strains displaying rifaximin resistance. There is developing worry that the percentage of resistance strains will increase in the future.[33] Rifaximin may also be used as an extension to vancomycin for treating patients with relapsing *C. difficile* infection. However, the quality of evidence of these studies was judged to be low. Although exposure to rifamycin may increase the risk of resistance in the future, it should be used for severe cases only.[33]

1.3.4 – Fidaxomicin

Fidaxomicin (Figure 1.5) is a macrocyclic anti-microbial agent which is explicitly utilized to treat CDI and was endorsed by the FDA in 2011. Fidaxomicin has an intricate structure containing an unsaturated macrolide with two sugar units connected; the molecule was isolated from the fermentation of *Dactylosporangium aurantiacum.*[22] Fidaxomicin is profoundly costly (~$156 USD/portion) and the chemical structure is patent protected.[25]

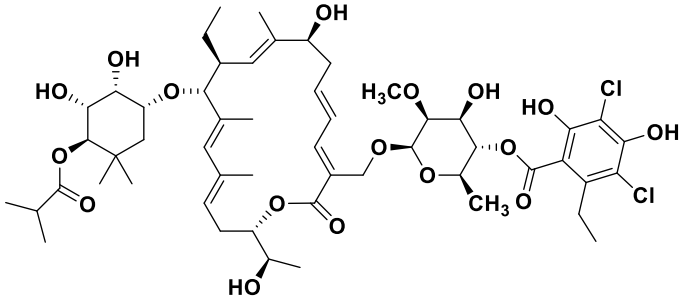


Figure 1.5 – Macrolide structure of fidaxomicin

Fidaxomicin showed antibacterial activities (MIC) in the range of 0.008 – 0.25 μg/mL for 90% of *C. difficile* isolates screened and also delivered promising results in CDI treatment.[22] Significantly, fidaxomicin displays narrow-spectrum antimicrobial potency; the medication specifically inhibits *C. difficile* and does not kill the useful GI microbiota.[20, 29] The rate of CDI recurrence was 22.6% for vancomycin and 11.7% for fidaxomicin.[30] Therefore, fidaxomicin better reduces the recurrence rate of CDI, when compared to vancomycin. The reason behind the lower rate of fidaxomicin recurrence is thought to be due to it allowing the commensal GI microbiome to remain unblemished while also inhibiting *C. difficile*.[30] These two properties are believed to be responsible for fidaxomicin's decreased rates of CDI recurrence.[29] Fidaxomicin inhibits RNA polymerase in bacteria where it binds to a site different from the rifamycin-type antibiotics.[29] The

significant cytotoxicity of fidaxomicin is not a major problem as its systemic absorption is negligible.[20] The medication shows the same level of tolerability as reference medications (i.e. vancomycin and rifaximin).[29] Fidaxomicin prevents harmful strains and diminishes the spore loads of *C. difficile* better than vancomycin. Studies are in progress to determine whether fidaxomicin can act as a prophylactic for CDI or as a post-antibiotic 'chaser' to inhibit antibiotic treatment-produced CDI.[25]

1.3.5 – Resistance of *C. difficile* to available medicines/drugs

C. difficile has shown lower vulnerability to the basic chemotherapeutics (vancomycin, metronidazole and fidaxomicin), however resistance has not been spread widely. For instance, *C. difficile* strains with lower susceptibility to metronidazole (MIC > 32 μg/mL) have been archived recently.[34] Furthermore, the Anaerobe Research Unit, UK - found four isolates of *C. difficile* with lower susceptibility to metronidazole while screening nearly 30 isolates.[34] This lower susceptibility has clinical noteworthiness due to oral administration of metronidazole (because of the high systemic absorption).[20] The lower susceptibility to metronidazole shown by *C. difficile* is not enduring as it is frequently lost after freeze-thawing or passaging of the bacteria.[20] The lower susceptibility to vancomycin (MIC - 4 μg/mL) and fidaxomicin (MIC - 6 μg/mL) has additionally been seen in *C. difficile* isolates.[20] The spread of *C. difficile* resistance is a developing issue and various new antibacterial agents are now being developed to battle against the *C. difficile* microorganisms.

1.4 – Development of CDI inhibitors

There are a few potential novel *C. difficile* inhibitors presently in different phases of advancement. Some of these CDI inhibitors will be described briefly with respect their potency, efficacy and clinical trials.

Nitazoxanide and derivatives

Nitazoxanide (Figure 1.6) is a nitrothiazolamide group compound that is utilized to treat intestinal parasites i.e. Cryptosporidium and Giardia. The drug was authorized by the FDA in 2004 and then eventually used for CDI treatment.[25, 39]

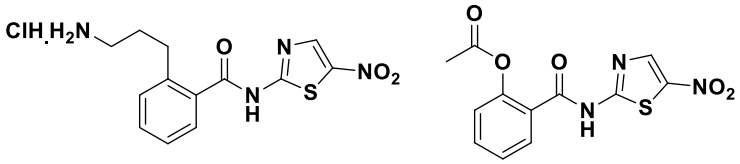


Figure 1.6 – Amixicile (left) and Nitazoxanide (right)

Nitazoxanide displayed antibacterial activities (MIC) in the range 0.3-1.0 µg/mL against *C. difficile*.[22] When the medication was first trialed against CDI, it was found to be less active than metronidazole and was shown to be effective in cases where metronidazole was not.[39, 41] Further tolerability investigations of nitazoxanide incorporated a correlation with vancomycin for a double-blind study and the outcomes indicated that vancomycin and nitazoxanide were similarly dynamic against CDI.[41] Nitazoxanide disrupts cell wall biosynthesis by repressing the pyruvate:ferredoxin oxidoreductase system.[22, 25] Nitazoxanide restrains the thiamine pyrophosphate nutrient co-factor of the pyruvate:ferredoxin oxidoreductase complex by competing with pyruvate

yet does not influence the complete chemical system. The pyruvate:ferredoxin oxidoreductase is a nonselective enzyme system for many beneficial microflorae in the gut but is a semi-selective enzyme system for the *C. difficile* target. The beneficial microflora makes use of pyruvate dehydrogenase enzyme system instead of utilizing the pyruvate:ferredoxin oxidoreductase enzyme system.[40] Nitazoxanide accumulates in the intestine before excretion due to its poor water solubility.[40]

Amixicile (Figure 1.6) is a hydrochloride salt structurally related to nitazoxanide. It was discovered from a library of 250+ nitazoxanide derivatives and its hydrochloride salt nature increased water solubility. The increased solubility helps in pyruvate:ferredoxin oxidoreductase inhibitory activity. Amixicile showed a superior survival rate (56%) compared to nitazoxanide (22%) and vancomycin (15%) in an optimized murine model of CDI studies. Amixicile and nitazoxanide have low CDI recurrence rates whereas vancomycin and fidaxomicin exhibited CDI recurrence in mice infected with CDI.[40] These promising results have confirmed that these nitrothiazolide derivatives are upcoming new CDI inhibitors and a few more investigations are required to consider if these two compounds are safe and more effective in the treatment of CDI.

Ridinilazole

Ridinilazole (formerly called - SMT19969) (Figure 1.7) is a dimer of benzimidazole with a pyridine linker that shows selectively antimicrobial potency against *C. difficile* but is not effective on any other Gram-positive and Gram-negative pathogens (including intestinal microflora). Ridinilazole exhibited antibacterial activities (MIC) in

the range of 0.125 – 0.25 µg/mL against several strains of *C. difficile*.[42, 43] The mechanism of action "needs to be fully ascertained" but the studies have declared that ridinilazole is involved in the inhibition of bacterial cell division – the medication is additionally known to diminish toxins and loads of *C. difficile* spores.[43]

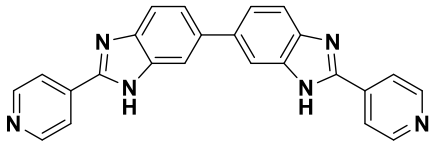


Figure 1.7 – Ridinilazole

Summit Pharmaceutical International has finished phase I clinical studies on ridinilazole and the results show excellent tolerability, low systemic absorption and specific focus on *C. difficile* without making collateral damage to the natural gut flora, and accordingly a decrease in the CDI recurrence rate. Additionally, ridinilazole passed supremacy criteria over vancomycin in multicentre phase II trials on CDI patients.[43] In preclinical studies, ridinilazole had established a strong bactericidal viability against all strains of *C. difficile* tested and Summit Pharmaceutical International is presently trialing this compound in phase III clinical trials.[43]

Surotomycin

Surotomycin (formerly known as CB-183,315; Figure 1.8) was discovered by Cubist Pharmaceuticals and developed by Merck & Co (who acquired Cubist Pharmaceuticals). This antibacterial agent is an orally administered, cyclic lipopeptide and is derived via semi-synthesis of the natural product daptomycin.[20] Surotomycin showed antibacterial activity (MIC) of 0.5 µg/mL against *C. difficile*. The efficacy and

safety studies of phase II clinical trials confirmed that surotomycin at two doses i.e. 125 mg/day or 250 mg/day was tolerable and at that dose the medication had a higher cure rate than vancomycin.[35]

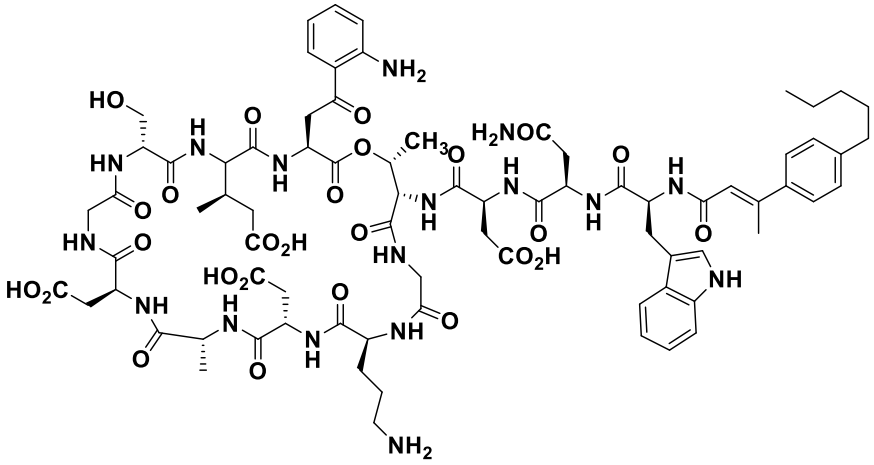


Figure 1.8 – Surotomycin

The CDI survival rate for surotomycin was (83.6%) better than that of vancomycin (79%).[36] Phase III clinical trials of this antibiotic were discontinued in 2017 due to its non-superiority to current therapies.[36]

Cadazolid

Cadazolid (Figure 1.9) is a crossover anti-microbial agent of the oxazolidinone class created by Actelion Pharmaceuticals. The structure of cadazolid is a hybrid of the known oxazolidinone and fluoroquinolone antibiotics. Cadazolid displayed antibacterial activities (MIC) in the range of 0.125 – 0.50 µg/mL against *C. difficile* isolates and obstructs biosynthesis of the bacterial cell wall. Cadazolid also restrains sporulation and toxin formation; moreover, cadazolid has low oral bioavailability and selectively shows potency against *C. difficile*, however it is benevolent to intestinal microflora.[44-45]

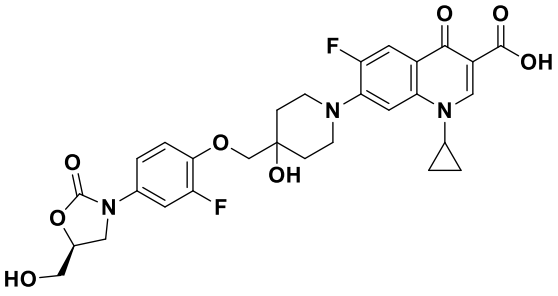


Figure 1.9 – Cadazolid

Cadazolid effectively finished phase I and II clinical trials with better efficacy and similar tolerability to vancomycin. The results of Phase III clinical trials concluded with mixed results but precise details were not disclosed.[46]

CRS3123

A novel diaryldiamine CRS3123 (previously REP3123; Figure 1.10) showed antibacterial activity (MIC) of 1.0 µg/mL against 108 clinical isolates of *C. difficile* and is presently under development with NIAID (National Institute for Allergy and Infectious Diseases).[22, 37] CRS3123 is a novel potent small molecule that inhibits methionyl-tRNA synthetase in *C. difficile* and also inhibits the production of toxins and spores.[37]

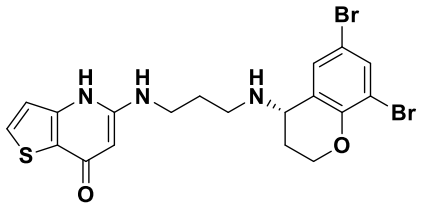


Figure 1.10 – CRS3123

C. difficile infection treatment with CRS3123 in a hamster model, resulted a significant improvement in survival rate when compared to vancomycin. Hamsters (62%-75%) treated with CRS3123 were alive at day 35 but all vancomycin-treated hamsters

were dead by day 17 (rate of dose 0.5 mg/kg and 5 mg/kg, respectively).[22] The phase I clinical trials of CRS3123 have been disclosed as overall well tolerated over a wide range of doses. This safety profile supports further investigation of CRS3123 as a treatment for CDI.[38]

1.5 – Development of binaphthylpeptide derivatives as an antibacterial agent

In our research laboratory, there has been a progressing research project focused on the discovery of novel antibacterial compounds initially around a binaphthylpeptide scaffold.[47, 48-54] The key moieties that grant antimicrobial potency to this group of compounds are displayed on the unique lead compound-**1**; **AVX-13616** (Figure 1.11). The basic characteristics of compound **1** includes a hydrophobic 1,1'-binaphthyl moiety, an isopentyl group connected to one of the naphthyl rings and a peptide back-bone connected to the other naphthyl ring. The peptide back-bone includes one to three amino acids (compound **1** has three), importantly one of the amino acids should contain a cationic (i.e. positively charged/basic) side chain for activity.

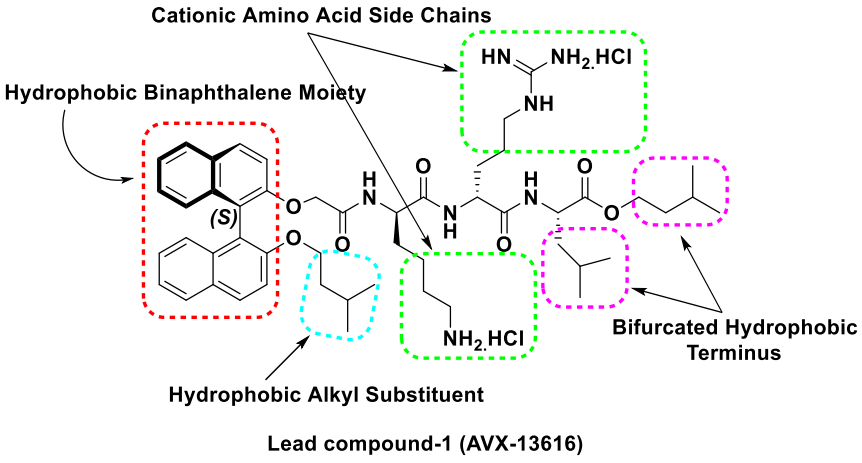
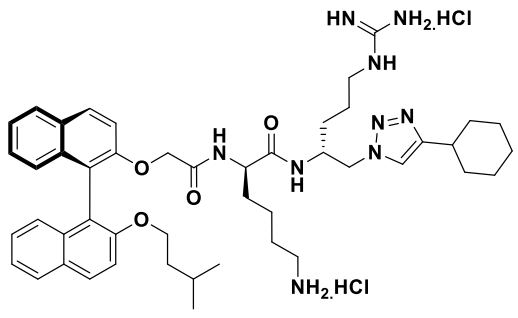


Figure 1.11 – Binaphthylpeptide compound (Lead compound **1**).

The amino acids arginine and lysine have been seen as an essential in providing an antimicrobial potency to this class of derivatives. The hydrophobic terminus is important for guaranteeing antimicrobial potency, despite the fact that bifurcation at this end does not appear to be vital.[47, 54] These binaphthylpeptide analogues for the most part show a wide range of Gram-positive antibacterial potency, for example AVX-13616 displayed antibacterial activities (MIC) of 2.0 µg/mL against VRSA and 4.0 µg/mL against VRE. Notably, AVX-13616 additionally showed antibacterial potency against *S. aureus* and MRSA strains that were resistant to linezolid. This compound showed decreased potency against Gram-negative microbes i.e. *K. pneumoniae* and *E. coli* with minimum inhibitory concentration (MIC) of >32.0 µg/mL and 16.0 µg/mL, respectively.[49] In 2010, compound-**1** (AVX-13616) was licensed to Valevia Pharmaceuticals and the organization planned to develop AVX-13616 as a medication in the form of creams, foams, gels, lotions and ointments for wound related infections of body surfaces such as skin and mucous membranes.[55] *In vitro* studies showed that resistance to the binaphthyl peptides grows gradually in contrast to vancomycin; for *S. aureus* 18 passages were required to induce vancomycin resistance, while AVX-13616 required in excess of 50 passages for *in vitro* resistance to develop.[49] Bacteria that inevitably evolved decreased vulnerability to AVX-13616 did not show cross-resistance to vancomycin.[49] These results demonstrated that vancomycin and AVX-13616 do not share the same mode of action. Generally, these peptide analogues are non-drug like as they have greater number of heteroatoms and their mono and dicationic nature gives them poor membrane permeability. Furthermore, recently synthesized analogues were not

appropriate for *in vivo* investigations on *C. difficile* infected mice because of their poor water solubilities; therefore, highlighting the need for more water-soluble derivatives.

Ongoing investigations showed that different 1,2,3-triazole analogues of compound **1** showed antimicrobial potency (MIC) in the range of 2.0 – 8.0 µg/mL against *C. difficile*.[47, 53] Lead derivatives **2** and **3** were synthesized as peptidomimetic analogues of compound **1** and these were found to show exceptional *in vitro* antimicrobial potency against both hypervirulent *C. difficile* and *S. aureus* (Figure 1.12 and 1.13).[54] In the *in vivo* murine model studies on CDI mice, compounds **2** and **3** showed some encouraging preliminary outcomes.[47] The current project aims to develop a library of novel derivatives around the lead compounds **2** and **3** for further SAR studies with endeavors being made to improve antimicrobial potency and selectivity for *C. difficile*.[56] Furthermore, it was planned to make compounds with improved water solubilities to aid oral dosing of compounds.

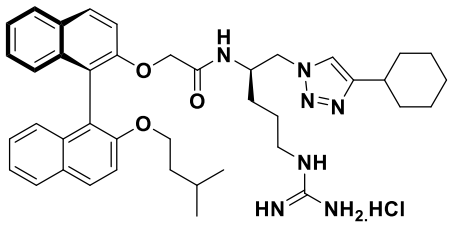


2

MIC values (µg/mL)

Bacterial Species	Compound-2	Vancomycin
S. aureus	4	**1**
MRSA	4	**1**
E. faecalis	2	**4**
S. pneumoniae	2	**1**
C. difficile	8	**0.5**
C. difficile **(RT027)**	8	**0.5**
E.Coli	8	**-**

Figure 1.12 – Lead compound **2** with a table of antibacterial activities against *S. aureus* (ATCC 29213), *MRSA* (NCTC 10442), *E. faecalis* (ATCC 29212), *S. pneumoniae* (ATCC 49619), *C. difficile* (ATCC 700057, *C. difficile* (RT027 – NSW132) and *E. coli* (ATCC25922).



3

MIC values (μg/mL)

Bacterial Species	Compound-3	Vancomycin
S. aureus	4	1
MRSA	2	1
E. faecalis	4	4
S. pneumoniae	8	1
C. difficile	16	0.5
C. difficile (RT027)	8	0.5
E.Coli	128	-

Figure 1.13 – Lead compound **3** with a table of antibacterial activities against *S. aureus* (ATCC 29213), *MRSA* (NCTC 10442), *E. faecalis* (ATCC 29212), *S. pneumoniae* (ATCC 49619), *C. difficile* (ATCC 700057, *C. difficile* (RT027 – NSW132) and *E. coli* (ATCC25922).

1.6 – Concepts and synthetic strategies for designing target compounds

1.6.1 – Incorporation of a peptidomimetic bioisostere

A previous researcher[47] in our group made analogues around compound **1** by substitution of the amide moiety (Figure 1.14a – blue circle) with a peptidomimetic bioisostere (i.e. 1,2,3-triazole ring; Figure 1.14c – blue segment). Previous investigations have affirmed that a 1,2,3-triazole ring is a suitable bioisostere for a typical peptide moiety as it shows a comparable planarity, dipole (Figure 1.14b and 1.14c) and spatial arrangement of polar atoms.[57-58] 1,4-Disubstituted-1,2,3-triazoles also have the possibility to donate and accept hydrogen bonds like normal amides.[58] Furthermore, they are viewed as more impervious to hydrolysis, oxidation and enzymatic cleavage than amides.

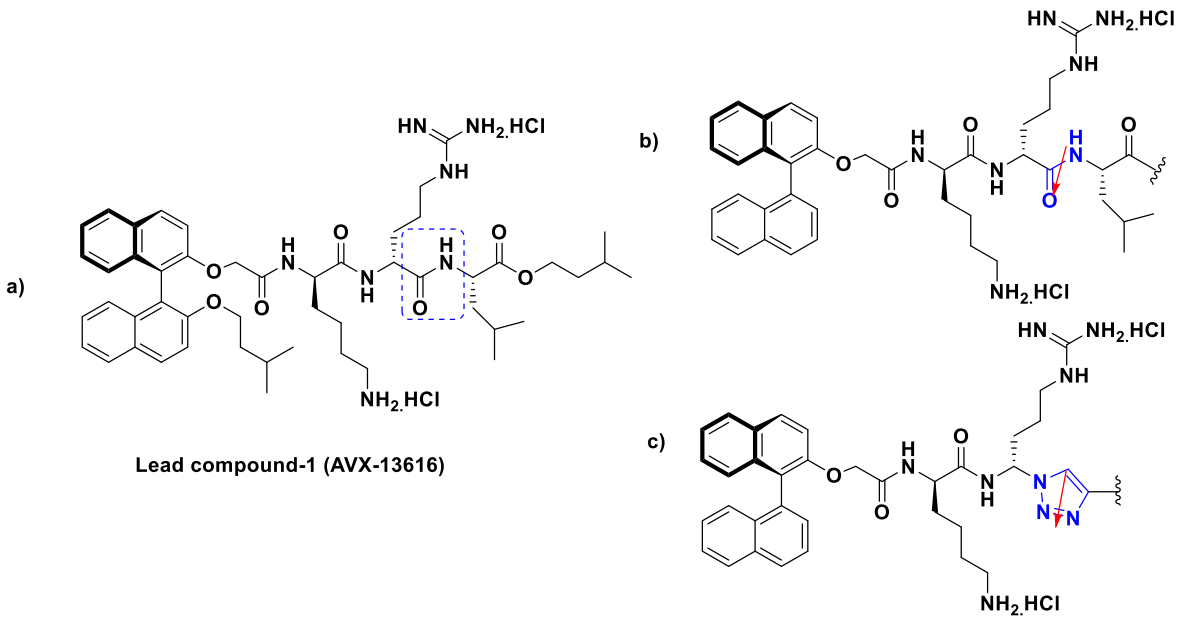
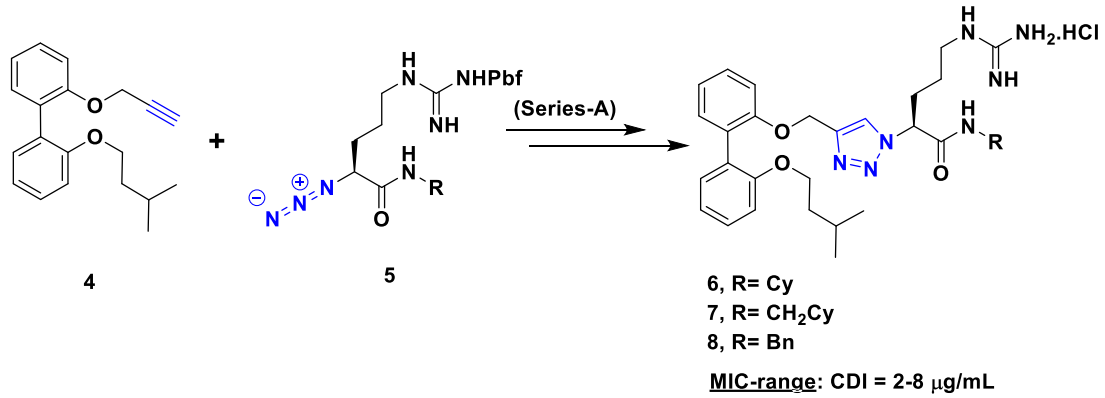
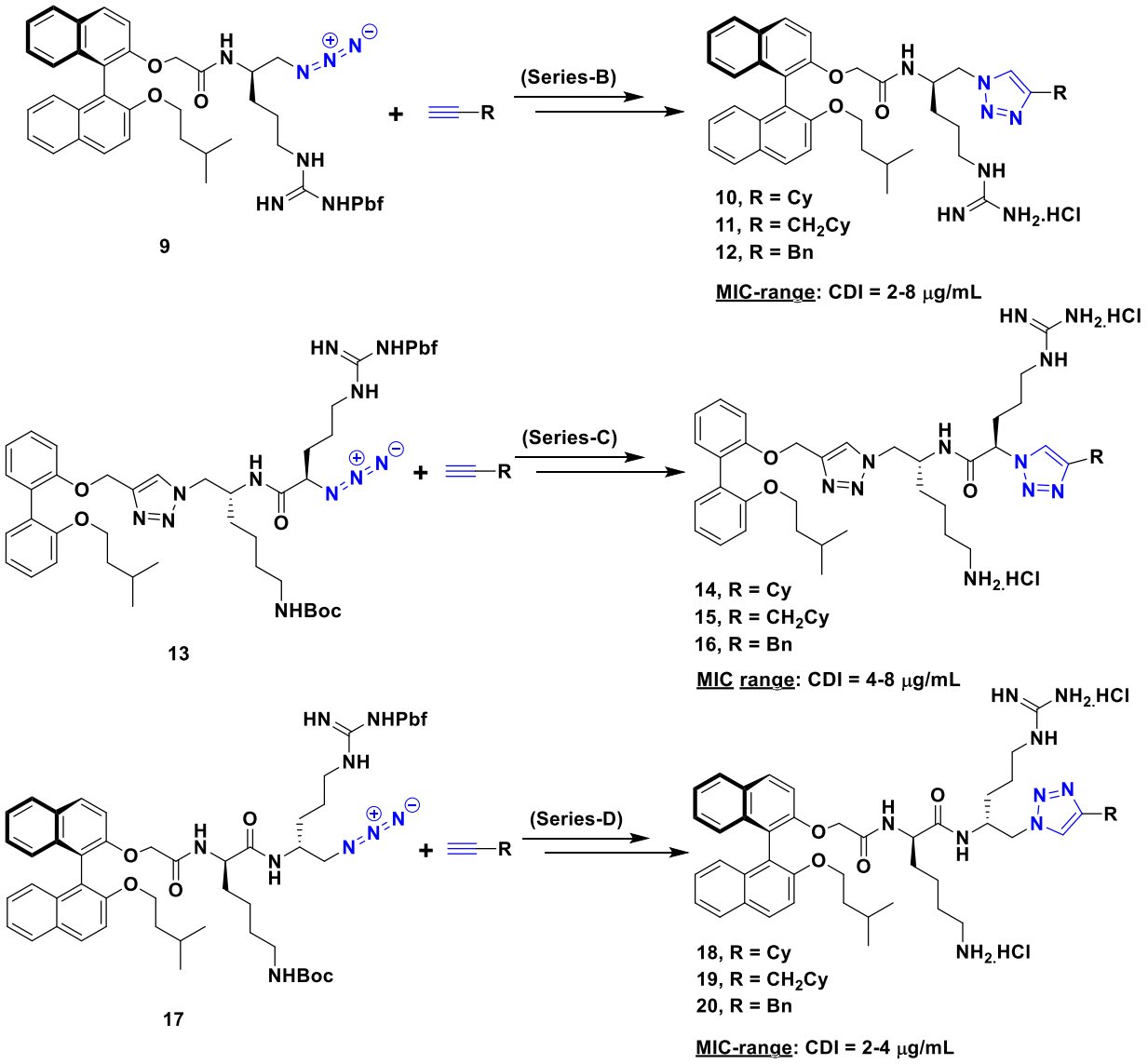


Figure 1.14 – **a)** Portion of lead compound **1** with targeted amide group (blue dotted rectangle)
b) Amide moiety (blue) and corresponding dipole moment (red)
c) 1,2,3-Triazole moiety (blue) and corresponding dipole moment (red).

In our laboratory, previous research has involved the replacement of a terminal amide (Figure 1.14a – blue circle) with a substituted 1,2,3-triazole moiety in the peptide chain of AVX-13616. The triazole moieties were installed *via* 'copper(I)-catalyzed alkyne-azide cycloaddition' (CuAAC) from the azide and alkyne precursors showed in Scheme 1.1.[57-59]



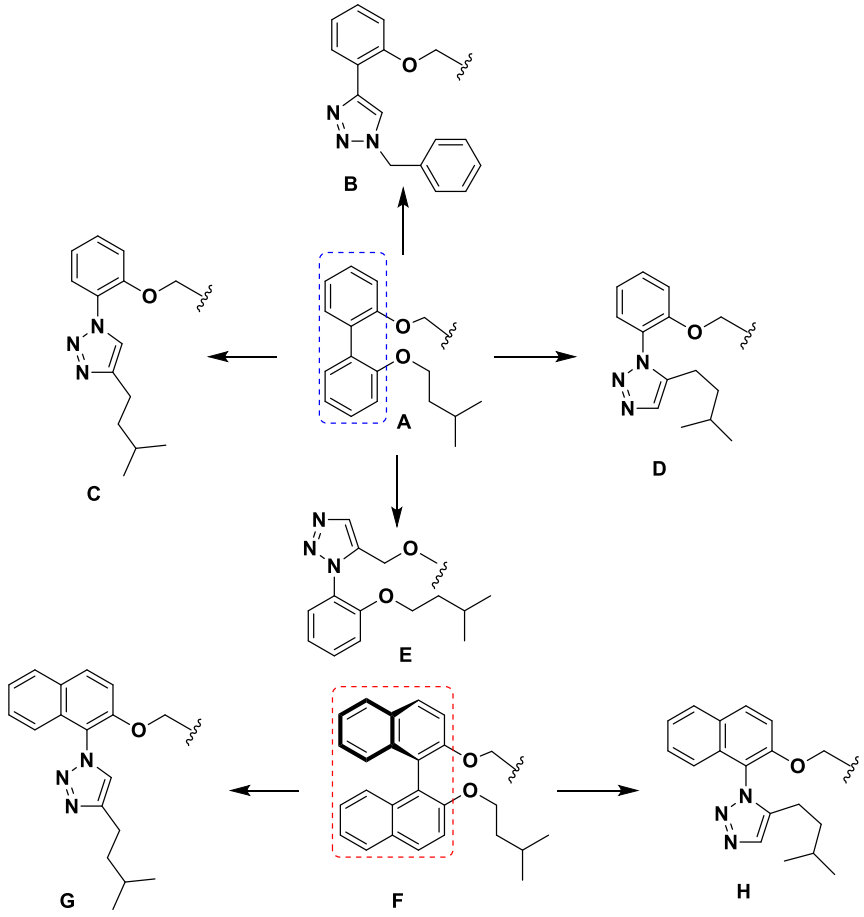


Scheme 1.1 – Previously synthesized triazole analogues showing their "click" chemistry precursors.

1.6.2 – Modification of the aromatic core

In this current project, it was planned to build upon these previous studies. A hydrophobic binaphthyl core **(F)** (red circle in Scheme 1.2) or its biphenyl analogue **(A)** (blue circle in Scheme 1.2) is mandatory for the antibacterial potency of AVX-13616 and its analogues. The biphenyl core **(A)** structure will be replaced by the *N*-phenyl-1,2,3-

triazoles **(B-E)** and the binaphthyl hydrophobic core **(F)** structure will be replaced by the *N*-naphthyl-1,2,3-triazoles **(G** and **H)**.



S**cheme 1.2** – Aromatic core replacement showing the changes from biphenyl core to phenyl-1,2,3-triazole cores and binaphthalene core to naphthalene-1,2,3-triazole cores.

These modifications were expected to provide peptide derivatives with better water solubilities than those previously synthesized due to the enhanced polarity of these 1,2,3-triazole derivatives.

1.6.3 – Design and synthesis of derivatives

The different *N*-phenyltriazole and *N*-naphthyltriazole peptide structures will be derivatized to provide the analogues **21 – 49** (Figure 1.15 – 1.16). These will be different

from each other based on two key structural parameters i.e., the aromatic core (Section 1.6.2), and the number and type of amino acids present in the peptide back-bone. In compounds **47 – 49**, the peptide moiety will be attached to the triazole group rather than the phenyl or naphthyl ring as in the other derivatives **21 – 46**. All these analogues **21 – 49** will be synthesized to allow structure-activity relationship (SAR) comparisons between specific analogues.

Past examination has demonstrated that monocationic derivatives can show valuable antibacterial potency;[47, 54] therefore, simple monocationic derivatives **21 – 34** will be targeted as well, because smaller and more synthetically accessible molecules are always preferable. Each synthesized scaffold will be derivatized by variation of the hydrophobic terminus (**R**) and Cu-catalysed 'click reactions' will be utilized for installation of the terminal 1,2,3-triazole moieties.

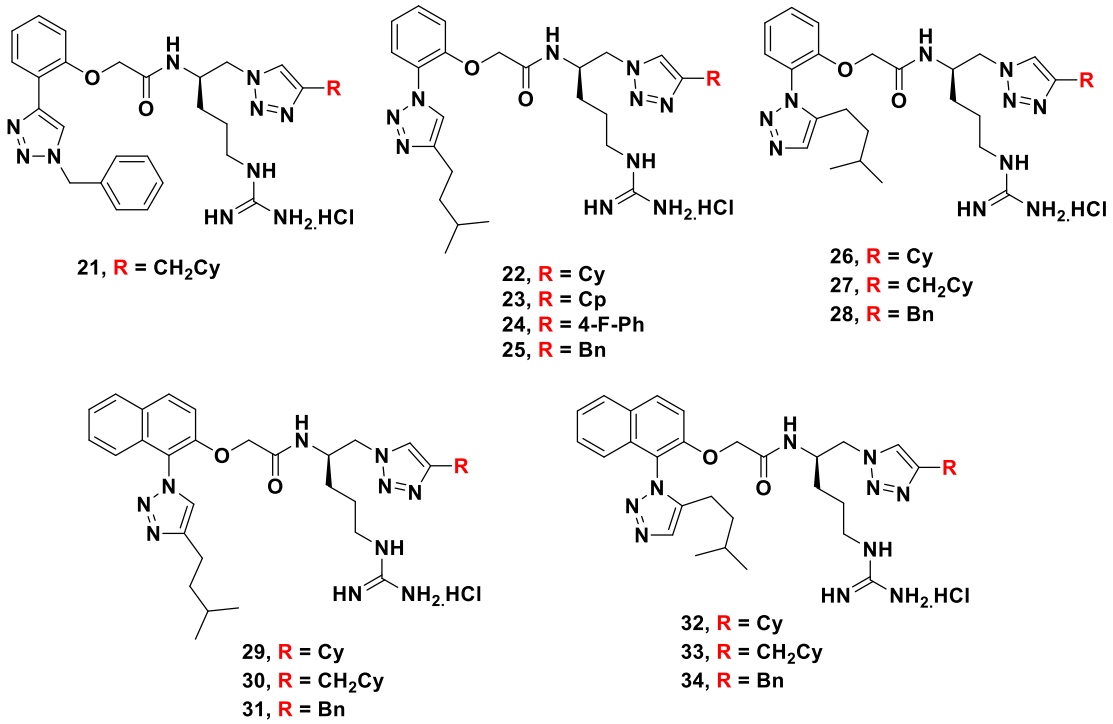


Figure 1.15 – Proposed mono-cationic derivatives **21 - 34** with variable hydrophobic structural elements (**R**)

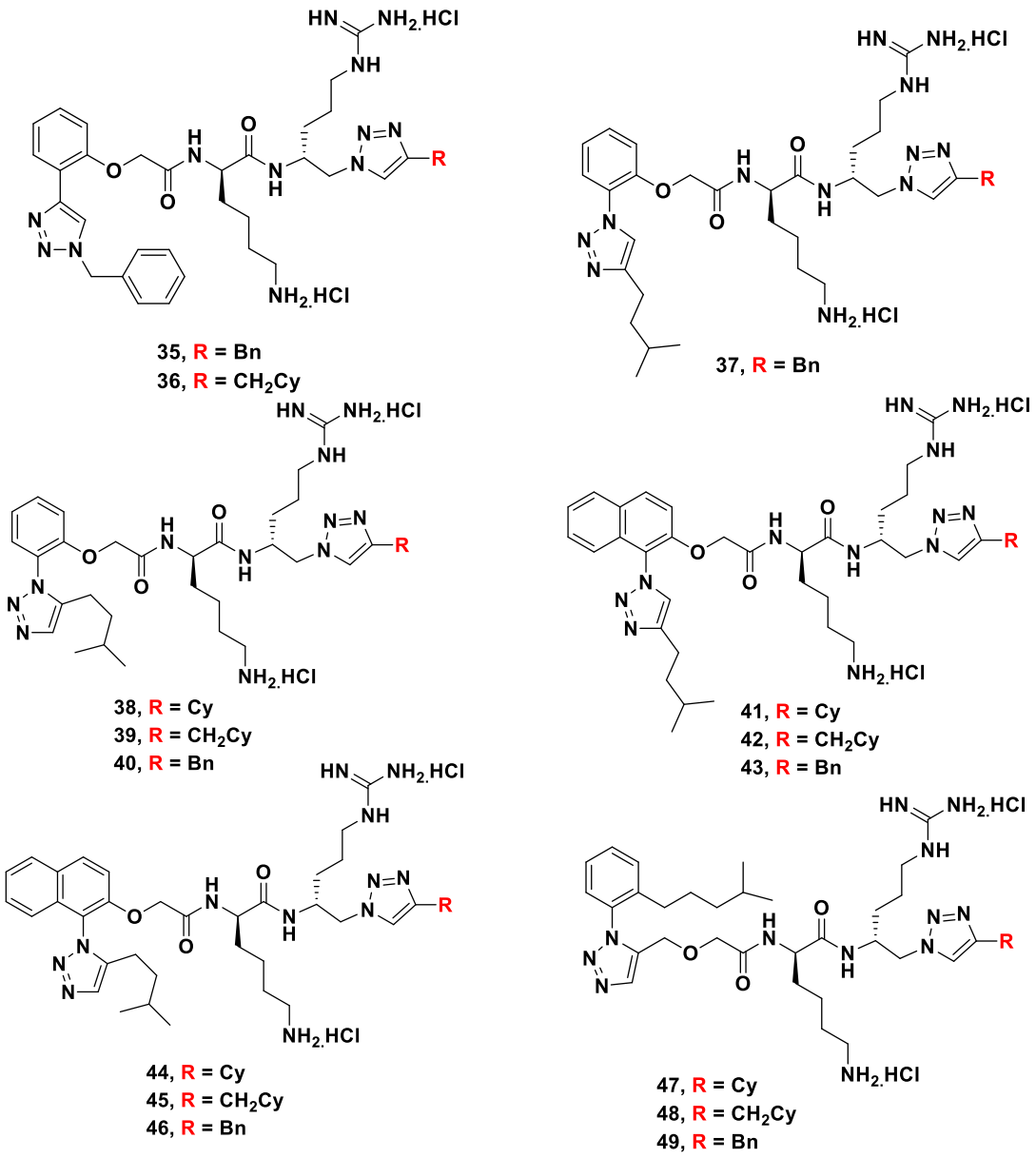


Figure 1.16 – Proposed di-cationic derivatives **35 - 49** with variable hydrophobic structural elements (**R**)

1.6.4 – Synthesis of analogues *via* a modular approach

To synthesize multiple analogue variations from a relatively simple synthetic route, a modular approach will be adopted. The different structural analogues **21 – 49** are initially linked by peptide bonds *via* peptide coupling reactions, then terminal triazole ring

formation *via* Cu-catalysed click reactions will allow formation of analogues from various building blocks (Figure 1.17) that hold these key reactive functional groups: amine (NH_2), acid (COOH), azide (N_3) and alkyne (C≡CH). The acid building block can be coupled to primary amines through formation of the required amide bonds. Furthermore, alkyne building blocks can be coupled with the requisite azide building blocks to construct the necessary 1,2,3-triazole moieties.

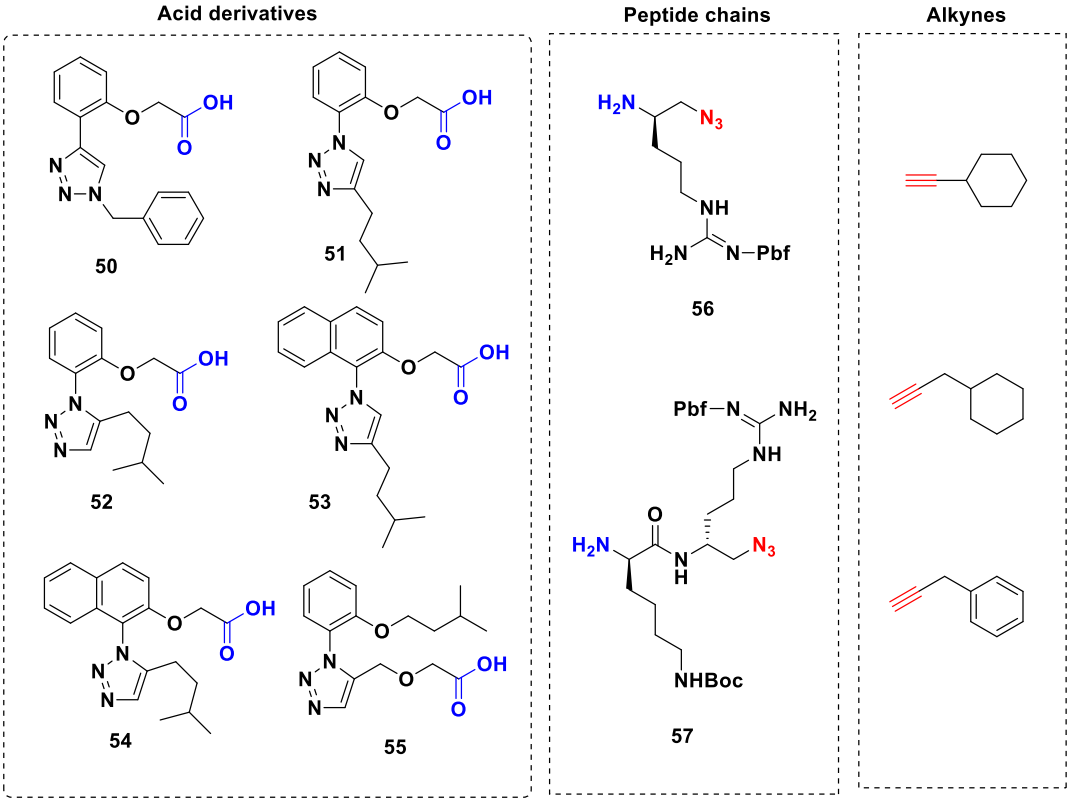
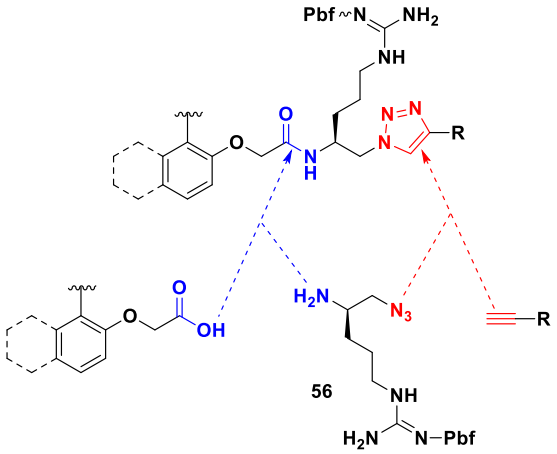


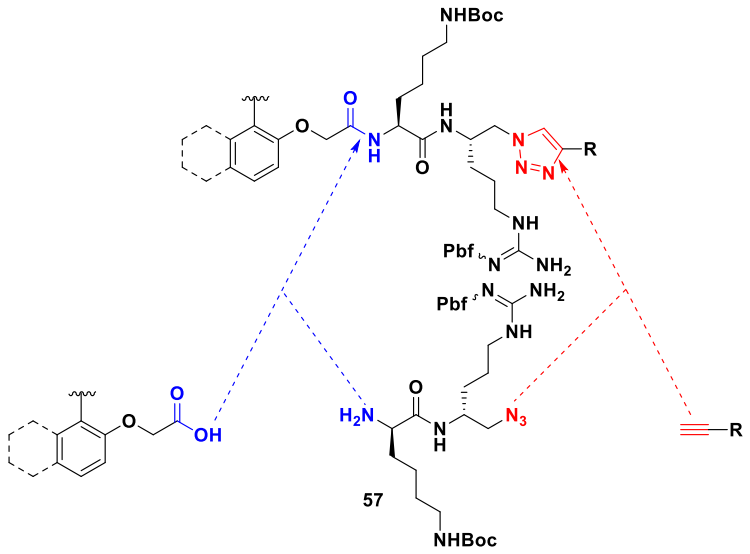
Figure 1.17 – Precursor building blocks for the modular approach to synthesis of analogues: the acid and amine moieties (blue) can be readily linked together *via* peptide coupling methods while the azide and alkyne functionalities (red) can also join together *via* CuAAC reactions.

Dual functionalization of the β-azidoamine **56** residue with both a group capable of peptide coupling (i.e. an acid) and a group capable of CuAAC reaction (i.e. an alkyne) allows ready access to monocationic analogues (Scheme 1.3).



Scheme 1.3 – Modular approach to synthesis of analogues: acid and amine functionalities (blue) are combined into an amide bond while the azide and alkyne functionalities (red) are coupled together *via* 1,2,3-triazole moiety

Dual functionalization of the β-azidoamine **57** residue with both a group capable of peptide coupling (i.e. an acid) and a group capable of CuAAC reaction (i.e. an alkyne) allows rapid access to dicationic analogues (Scheme 1.4).



Scheme 1.4 – Modular approach to synthesis of analogues: acid and amine functionalities (blue) are combined into an amide bond while the azide and alkyne functionalities (red) are coupled together *via* 1,2,3-triazole moiety.

1.6.5 – Proposed synthetic routes and challenges

The previously mentioned forerunner building blocks (Figure 1.17) will be synthesized from known and new synthetic precursors. The previously mentioned analogues (section 1.6.3) will be accomplished from peptide coupling and CuAAC reactions followed by side-chain N-deprotection and HCl salting. This iterative derivatization of the different scaffold types will facilitate the formation of a library of novel antibacterial derivatives. Most importantly, the proposed synthesis will require three protecting groups i.e. Boc (**I**), Pbf (**J**) and Fmoc (**K**) (Figure 1.18). These protecting groups are utilized for the protection of nucleophilic nitrogen atoms.

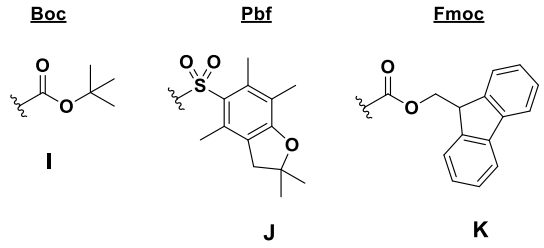
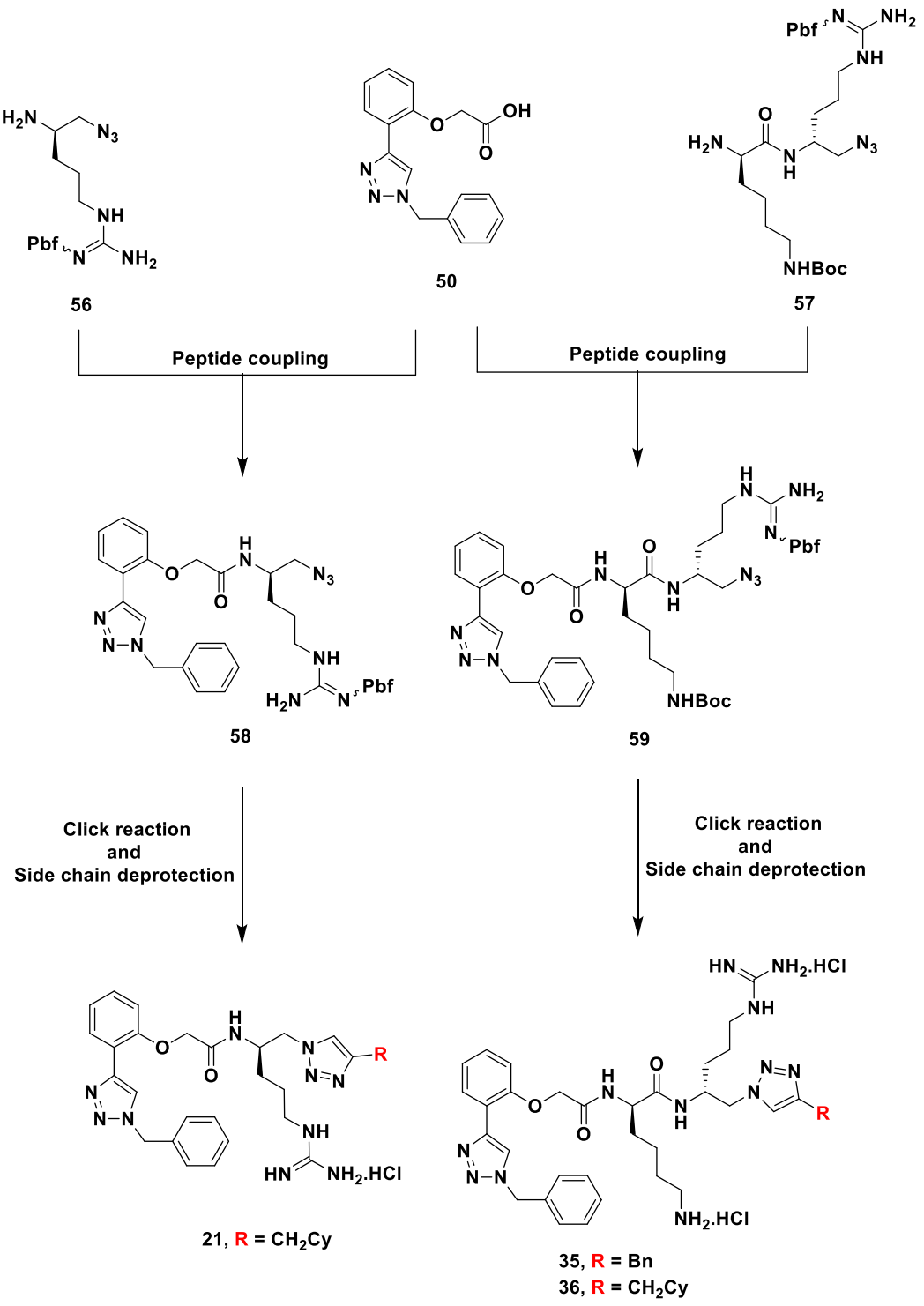


Figure 1.18 – Nitrogen protecting group structures and abbreviations

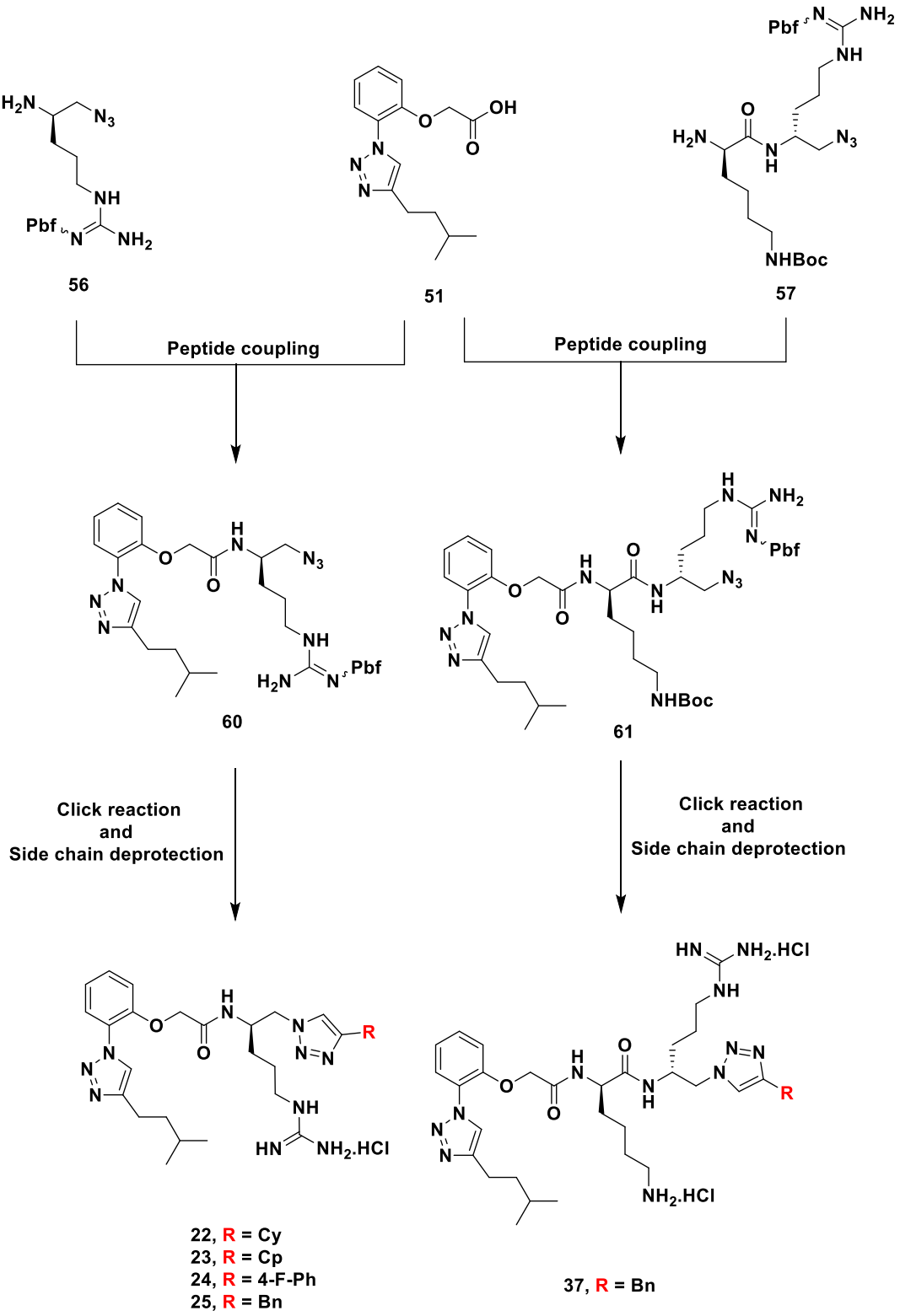
Boc and Fmoc protecting groups can be utilized to protect primary amines as carbamates and the Pbf protecting group can be utilized to protect guanidino residues *via* formation of an arylsulfonimide. Boc protected lysine and Pbf protected arginine amino acid sidechains can be utilized, and these two protecting groups can be deprotected together in the last step of a reaction sequence which permits the side chains to be unreactive throughout the entire synthetic process except for the last step. The Fmoc group will be used in known synthetic strategies for building the unprotected β-azidoamine precursors **56** and **57**.[60]

The designed synthetic routes (Schemes 1.5 – 1.10) will permit the divergent synthesis of numerous scaffolds from straightforward precursors. This synthetic strategy will allow various scaffold varieties to be prepared from the key precursor building blocks, which can be derivatized to generate a number of potential antibacterial products.

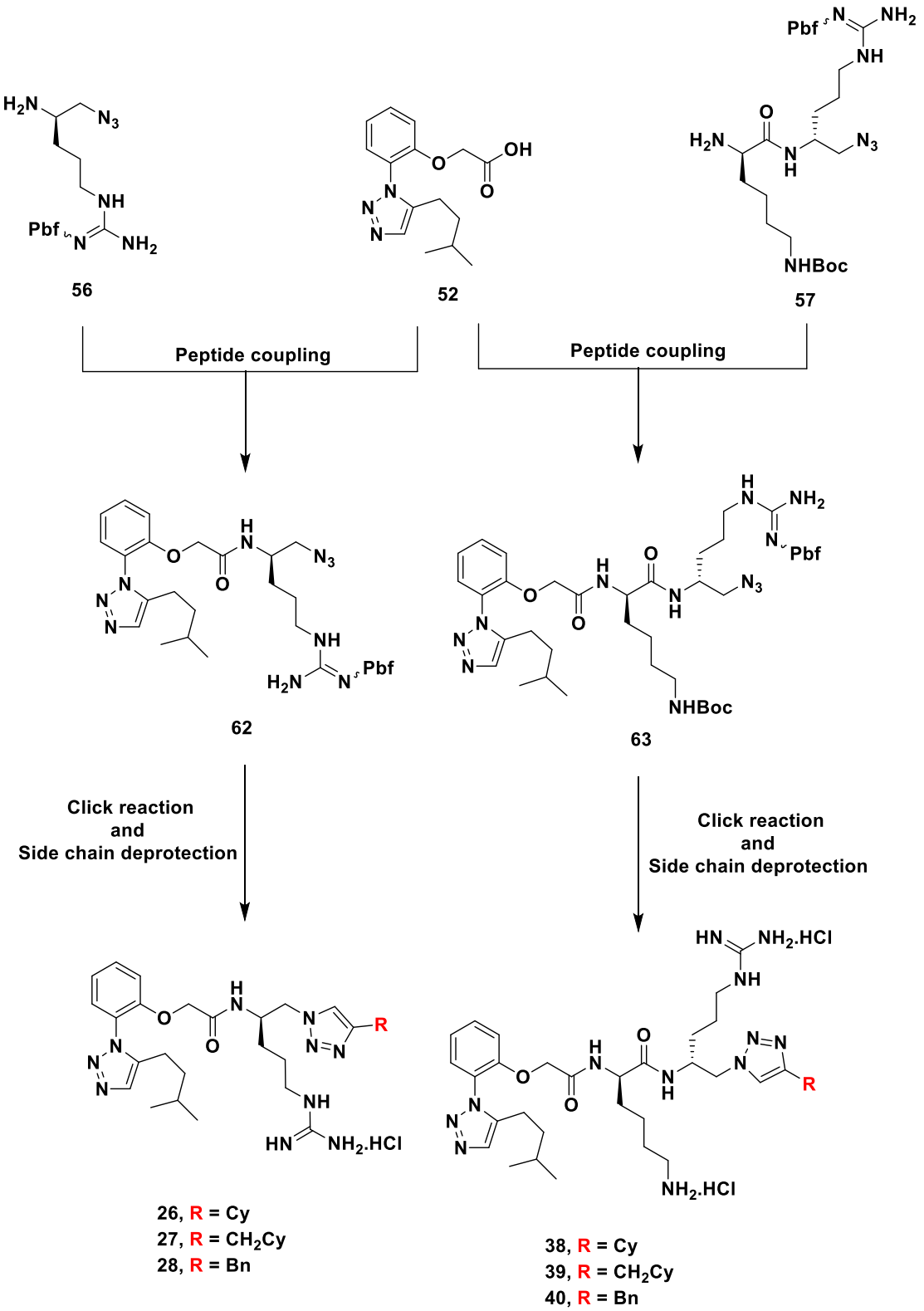
Schemes 1.5 – 1.10 employ the same pairs of β-azidoamines (**56** and **57**) coupling partners with the different acids **50 – 54**. Scheme 1.11 illustrates the β-azidoamine **57** coupled to the acid **55** resulting in products **47 – 49** in which the peptide side chain is attached to the triazole moiety.



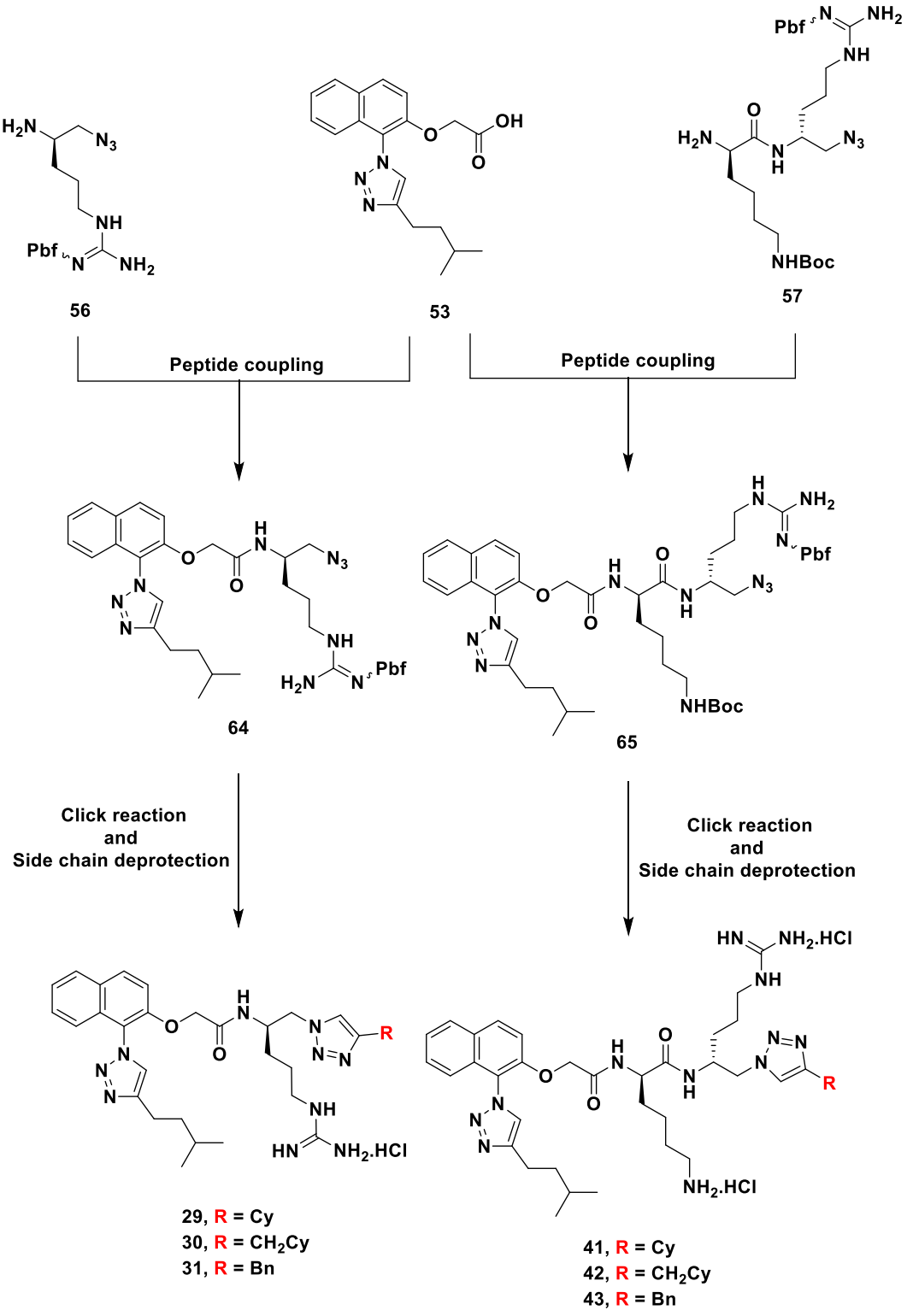
Scheme 1.5 – Potential synthetic pathway for analogues **21**, **35** and **36**



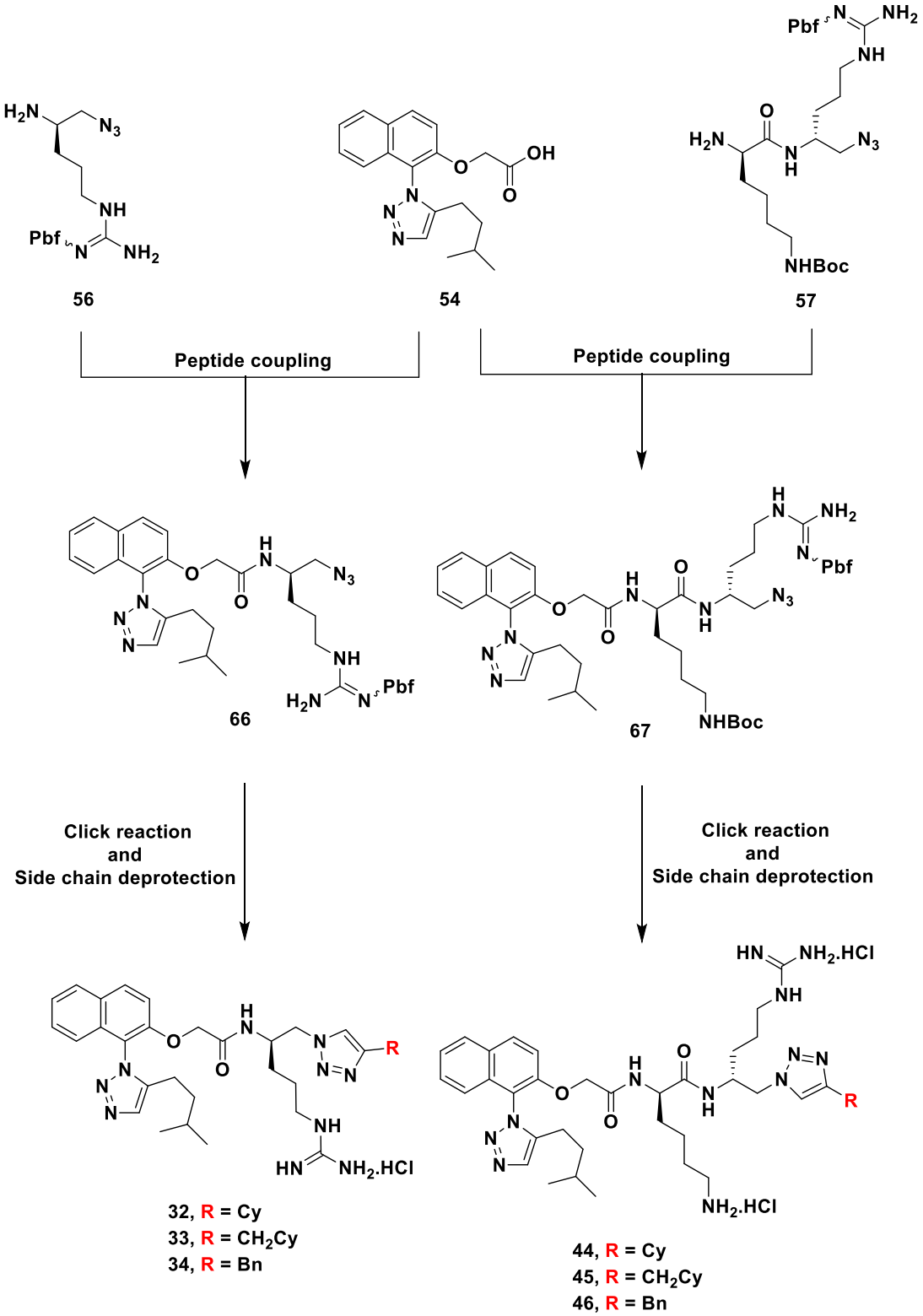
Scheme 1.6 – Potential synthetic pathway for analogues **22 - 25** and **37**



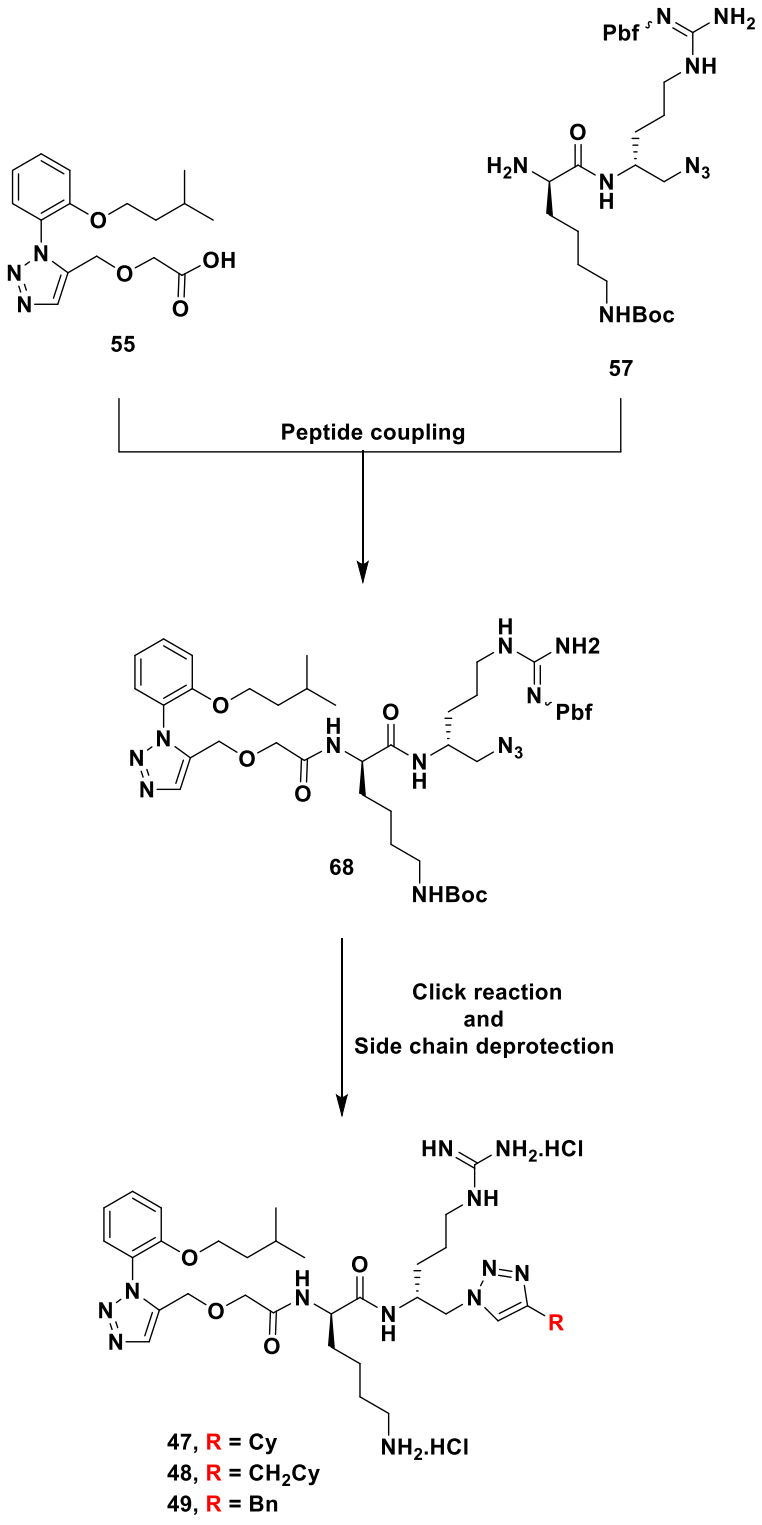
Scheme 1.7 – Potential synthetic pathway for analogues **26 – 28** and **38 - 40**



Scheme 1.8 – Potential synthetic pathway for analogues **29 - 31** and **41 – 43**



Scheme 1.9 – Potential synthetic pathway for analogues **32 - 34** and **44 - 46**



Scheme 1.10 – Potential synthetic pathway for analogues **47 - 49**

1.7 – Project Aims

It is an important medical requirement to develop novel antibacterial compounds to effectively treat CDI. Therefore, a joint research project (NHRMC Project; Grant No. APP1124032) was established between the University of Wollongong (drug design and synthesis), the University of Western Australia (microbiology) and Monash University (pharmacology) to synthesize and develop potent CDI chemotherapeutics. The aims of this project are:

- To incorporate a more polar hydrophilic moiety (i.e. a 1,2,3-triazole moiety) into the hydrophobic region of the lead binaphthylpeptides **2** and **3** to increase gastrointestinal stability and solubility.
- To modify the hydrophobic binaphthyl core of **2** and **3** by making *N*-phenyl-1,2,3-triazole and *N*-naphthyl-1,2,3-triazole derivatives in an attempt to increase compound solubility.
- To synthesize both dicationic and monocationic peptidomimetics.
- To design and establish a viable and scalable synthesis of several novel triazole-containing scaffolds.
- To ascertain the antimicrobial activity of the synthesized derivatives against Gram-positive bacteria, Gram-negative bacteria and fungi *via* MIC assays.
- To develop and utilize a comparative solubility assay for determining the relative water solubilities of the synthesized compounds.
- To ascertain the general toxicity of the synthesized compounds *via* cytotoxicity assays.

- To ascertain an efficacy of one or more compounds as a CDI chemotherapeutic *via* testing in an *in vivo* CDI mouse model.
- To design and identify a novel hit compound that could be utilized in the effective treatment of CDI.

  and
- To explore the structure-activity relationships of the synthesized compounds by utilizing the obtained MIC data.

The knowledge gained from this project would contribute to our understanding of the cationic phenyl and naphthyl peptide antibacterial class of compounds.

2.0 – Synthesis: results and discussion

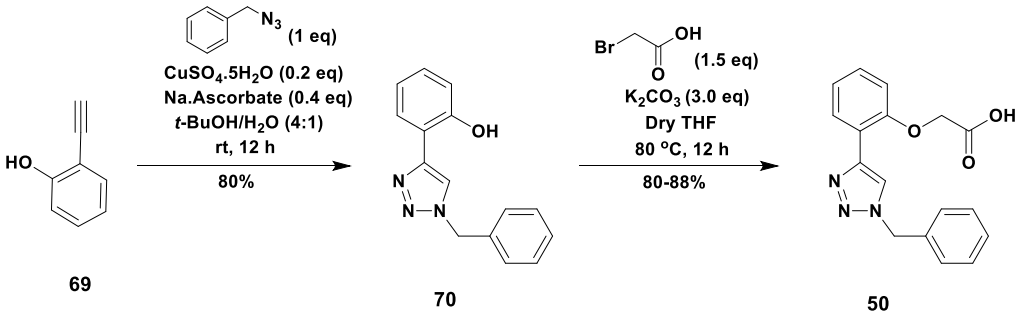
2.1 – Synthesis of precursor building blocks

This chapter describes the synthesis of the proposed targeted antibacterial derivatives from commercially available precursors. The known compounds are noted with literature references.

2.1.1 – Synthesis of the aromatic core structures

2.1.1.1 – Synthesis of the carboxylic acid 50

The synthesis and incorporation of a 1,4-disubstituted-1,2,3-triazole ring in drug like molecules has become a widespread practice and the common 'click reaction' [i.e., a Cu-catalysed azide-alkyne cycloaddition (CuAAC)] is the most reliable methodology for the formation of these moieties.[57–59] Therefore, the strategy for the formation of the key intermediate **50** (Scheme 2.1) followed a two-step process of a 'click reaction' followed by *O*-alkylation.



Scheme 2.1 – Synthesis of acid **50** from the alkyne **69** *via* Cu-catalysed [3 + 2] cycloaddition reaction between alkyne **69** and benzyl azide, followed by *O*-alkylation of **70** with bromoacetic acid to obtain target acid **50**.

The reaction of benzylazide with the alkyne **69** in the presence of Cu(II)/sodium ascorbate in *t*-BuOH/H_2O for 12 h at rt gave the triazole **70** in 80% yield after purification by flash column chromatography. Analysis of the ^{1}H NMR spectrum of the triazole **70**

revealed a characteristic singlet resonance for the triazole proton at δ_H 7.72. The molecular structure of compound **70** was further verified by the presence of an ion peak at *m/z* 252.1136 in the HRMS (ESI) that was assigned to the protonated molecular ion ([M + H]$^+$) (calculated for $C_{15}H_{14}N_3O$ 252.1137).

The base-promoted *O*-alkylation of **70** with bromoacetic acid was achieved using K_2CO_3 in dry THF at reflux (Scheme 2.1) and after acidification produced acid **50** in 80% yield. This reaction sequence was also effective on a larger scale with an 88% yield of **50** obtained starting with 2.00 g of the precursor alkyne **69**.

Analysis of the ^{1}H NMR spectrum of acid **50** revealed a characteristic singlet resonance at δ_H 8.46 for the triazole proton. The C-5 carbon of the triazole ring resonated at δ_C 123.8 and was assigned from the gHSQC correlation of the triazole proton (δ_H 8.46) to this ^{13}C NMR resonance (Figure 2.1).

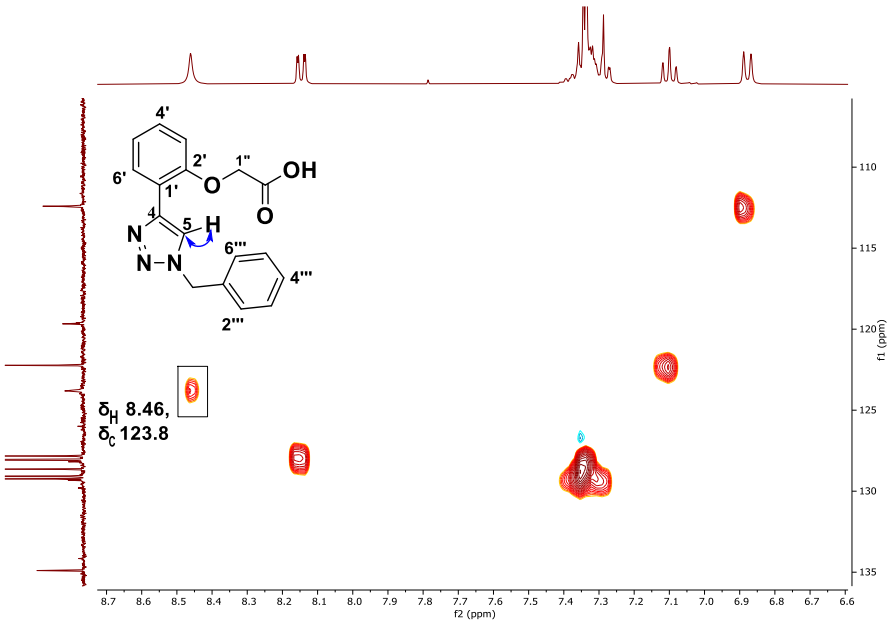


Figure 2.1: gHSQC spectrum of compound **50** (400 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-5 is highlighted.

Analysis of the gHMBC spectrum of **50** showed the resonance at δ_C 143.8 correlated to δ_H 8.46 and was assigned to the quaternary carbon of the triazole. Further the resonance at δ_C 170.8 was assigned to the carbonyl carbon of the carboxylic acid group as it correlated to the methylene protons (δ_H 4.70) of the carboxylic acid side chain (Figure 2.2).

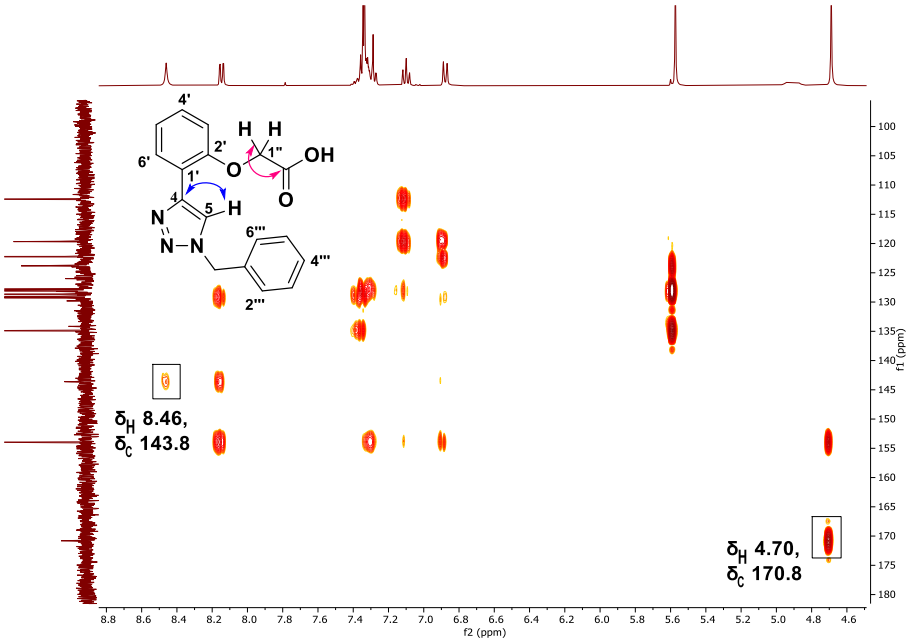


Figure 2.2: gHMBC spectrum of compound **50** (400 MHz, $CDCl_3$)**.** The two-bond correlations between the triazole proton and C-4, H-1" and carbonyl carbon of COOH are highlighted.

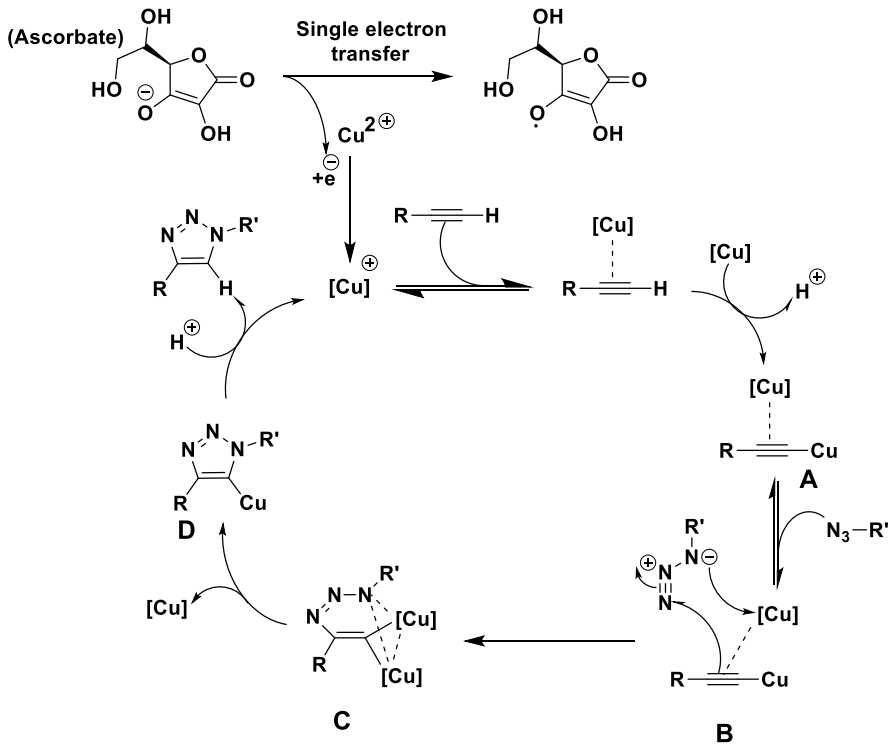
The molecular structure of compound **50** was further verified by the presence of the ion peak at *m/z* 332.1003 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{17}H_{15}N_3O_3Na$ 332.1011).

Mechanism of the CuAAC reaction:

The CuAAC reaction mechanism (Scheme 2.2) starts with the addition of a Cu(I) catalyst that can be added directly or prepared *in situ* from a Cu(II) salt by reduction using excess equivalents of sodium ascorbate (a single electron transfer reagent).[59] The sodium

ascorbate avoids oxidation of the active Cu(I) catalytic species to the inactive Cu(II) species.[62-63] This allows the reaction to be performed in an open-air environment without the requirement for an inert atmosphere.

The active catalytic species Cu(I) reacts with the Cu-π bonded alkyne to form the copper acetylide **A**. The π-bound copper of the copper acetylide **A** coordinates with the azide to form the azido copper acetylide **B**. Then first nitrogen of the azido copper acetylide **B** coordinates with the π-bound copper to form a six-membered copper metallacycle **C**. The 2nd copper atom acts as a stabilizing donor ligand and ring contraction results in the triazolyl-copper derivative **D** that further undergoes protonolysis to deliver the triazole product and closes the catalytic cycle.

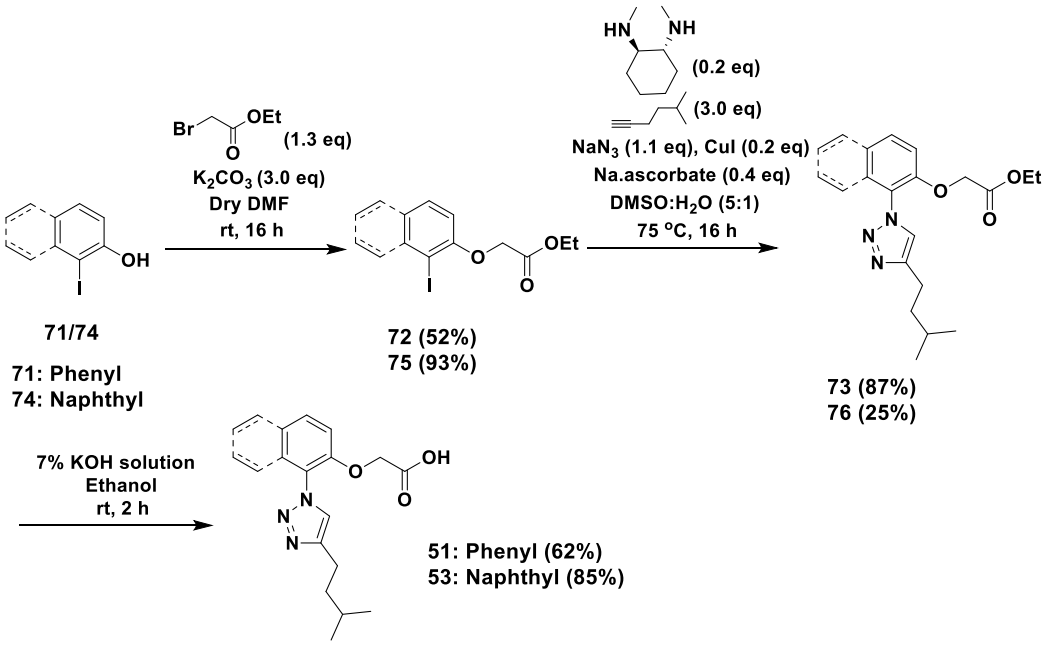


Scheme 2.2 – Proposed catalytic cycle for the CuACC reaction: [Cu] represents a Cu(I) complex that is coordinated to the requisite number of ancillary ligands. The mechanism allows for control over the regioselectivity of the cycloaddition by restricting the relative orientation of the 1,4-substituents.

The regioselectivity for the 1,4-disubstituted-1,2,3-triazole product is controlled by the binding orientation of the azide and alkyne components to the active Cu(I) complex, which controls the orientation of the resultant [3 + 2]-cycloaddition.[59, 62-63] This orientation mechanism was confirmed by multiple CuAAC studies that included kinetic analysis and DFT calculations.[59, 62-65] Kinetic analysis confirmed that the reaction is 2nd order with respect to the concentration of Cu(I); this involves the prospective role of a di-nuclear acetylide-bridged Cu(I) complex as an active species[62-63] (Scheme 2.2). Notably, the mild catalytic reaction conditions produced consistent, clean and high-yielding reactions for the preparation of 1,4-disubstituted-1,2,3-triazoles.

2.1.1.2 – Synthesis of the carboxylic acids 51 and 53

The synthesis of acids **51** and **53** from their respective precursors **71** and **74** was accomplished in three steps: (i) an *O*-alkylation, (ii) a one pot process of azidation and a CuAAC reaction, and (iii) an ester hydrolysis of **73** and **76** using base which produced, after acidification, the acids **51** and **53**, respectively (Scheme 2.3).



Scheme 2.3 – Synthesis of acid **51** and **53** from respective alcohol precursor **71** and **74** *via* *O*-alkylation of alcohol **71** and **74** to give iodo ester **72** and **75**, followed by a one pot (azidation/click reaction) synthesis of **73** and **76**, then ester hydrolysis of **73** and **76** to furnish target acids **51** and **53**.

The base-promoted *O*-alkylation reaction of **71** and **74** with ethyl bromoacetate produced the ethers **72** and **75** in yields of 52% and 93%, respectively (Scheme 2.3). The known ether **72** exhibited spectroscopic data that were in agreement with those values reported previously.[78] The intermediates **72** and **75** were then treated with 5-methyl-1-hexyne in the presence of CuI/NaN_3/Na.ascorbate and racemic *trans-N,N'*-dimethylcyclohexane-1,2-diamine in DMSO and water (5:1) at 75 °C and gave esters **73** and **76** in yields of 87% and 25%, respectively. In this one-pot azidation and copper catalyzed click reaction process, the diamine ligand assists in the azidation reaction by acting as a ligand for copper(I) or (II). The copper(I) then catalyzes the subsequent CuAAC reaction. Analysis of the ^{1}H NMR spectra of esters **73** and **76** revealed characteristic singlet resonances at δ_H 8.16 and 7.67, respectively, assigned to the

corresponding 1,2,3-triazole protons. The molecular structures of compounds **73** and **76** were further confirmed by the presence of the ion peaks at *m/z* 318.1831 and *m/z* 368.1985, respectively, in the HRMS (ESI) assigned to the protonated molecular ions ([M + H]$^+$) (calculated for $C_{17}H_{24}N_3O_3$ 318.1818 and $C_{21}H_{26}N_3O_3$ 368.1974, respectively).

The target acid **51** was realized in a yield of 62% from hydrolysis of the ester **73** with 7% KOH solution in ethanol. The ^{1}H NMR spectrum of **51** showed the characteristic resonance for the triazole proton at δ_H 8.20 (s, 1H). The C-5 carbon of the triazole ring resonated at δ_C 123.5 and was assigned by analysis of the gHSQC spectrum which showed a correlation of this resonance to the triazole proton (δ_H 8.20) (Figure 2.3).

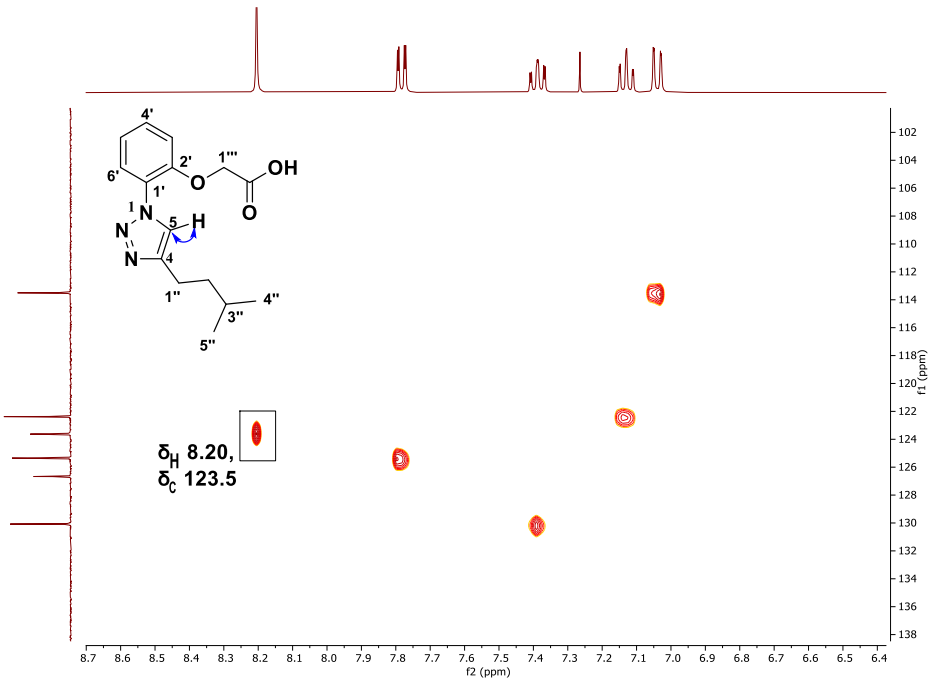


Figure 2.3: gHSQC spectrum of compound **51** (400 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-5 is highlighted.

The gHMBC spectrum analysis revealed that the resonance at δ_C 147.6 correlated to δ_H 8.20 and was assigned to the quaternary carbon (C-4) of the triazole.

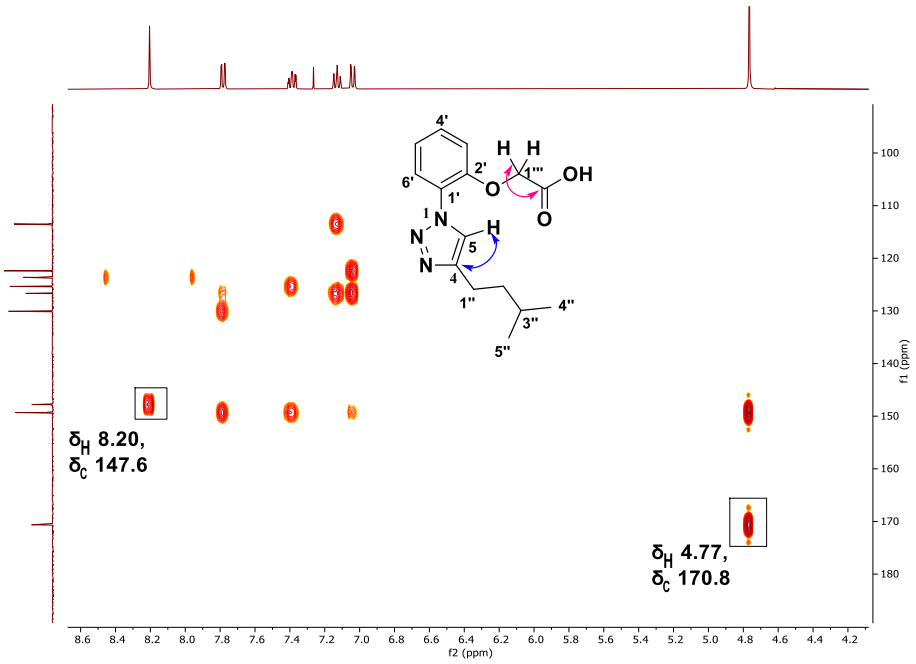


Figure 2.4: gHMBC spectrum of compound **51** (400 MHz, $CDCl_3$)**.** The two-bond correlations between the triazole proton and C-4, H-1''' and carbonyl carbon of COOH are highlighted.

Further the resonance at δ_C 170.8 was assigned to the carbonyl carbon of carboxylic acid group as it correlated to the methylene protons (δ_H 4.77) of the acid side chain (Figure 2.4). The molecular structure of compound **51** was further established by the presence of an ion peak at *m/z* 290.1513 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{15}H_{20}N_3O_3$ 290.1505).

The desired carboxylic acid **53** was realized in a yield of 85% from the hydrolysis of the ester **76** using 7% KOH solution in ethanol. The ^{1}H NMR spectrum of **53** showed the characteristic singlet resonance of triazole proton at δ_H 7.67 which was correlated in the gHSQC spectrum to the resonance at δ_C 124.6 which was assigned to C-5 (Figure 2.5).

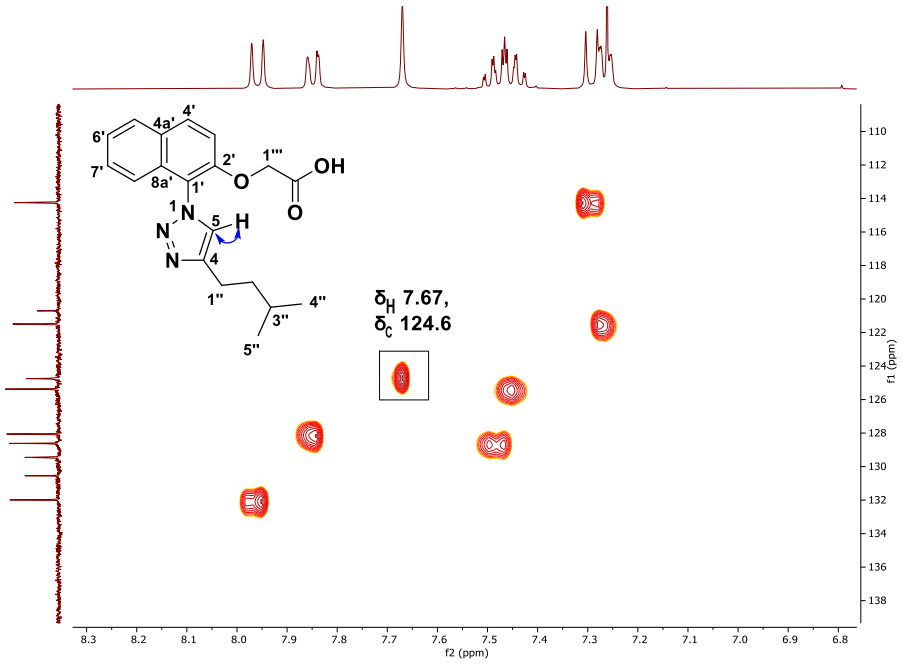


Figure 2.5: gHSQC spectrum of compound **53** (400 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-5 is highlighted.

In the gHMBC spectrum, the resonance at δ_H 7.67 correlated to the resonance at δ_C 148.0 for C-4 (Figure 2.6), while the resonance at δ_C 170.4 was assigned to the carbonyl carbon of the carboxylic acid side chain as it correlated to the methylene protons that resonated at δ_H 4.74 (Figure 2.6).

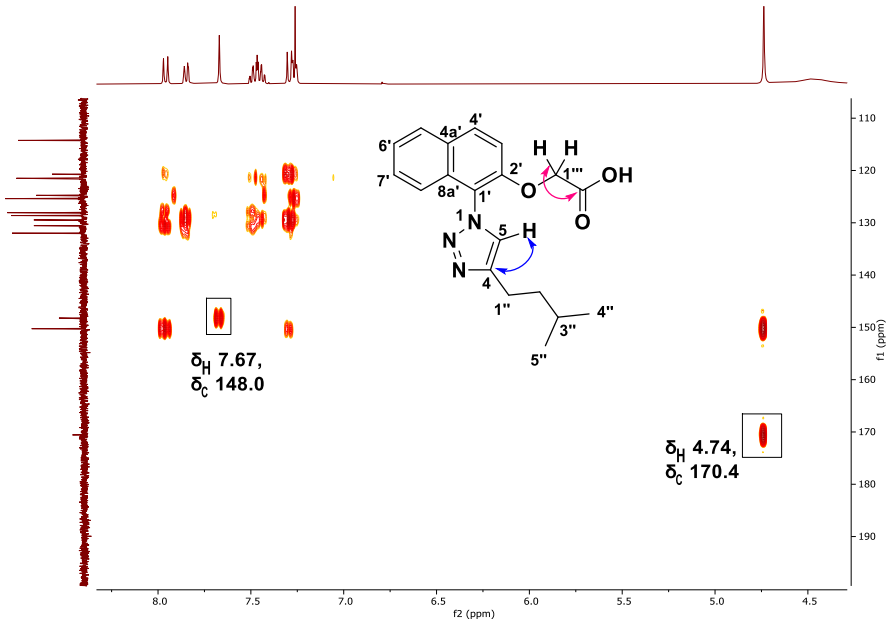
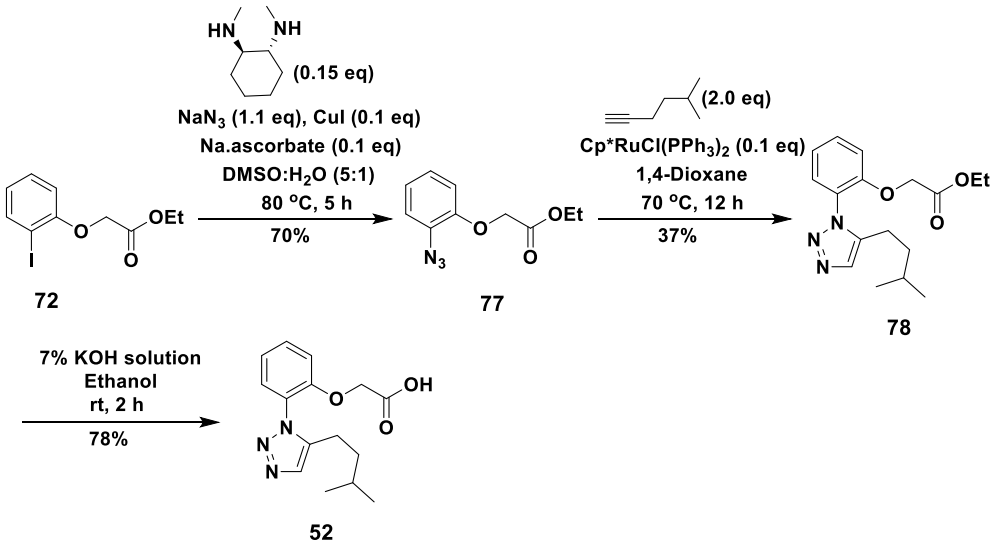


Figure 2.6: gHMBC spectrum of compound **53** (400 MHz, $CDCl_3$). The two-bond correlations between the triazole proton and C-4, H-1''' and carbonyl carbon of COOH are highlighted.

The molecular structure of compound **53** was further verified by the presence of an ion peak at *m/z* 340.1667 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{19}H_{22}N_3O_3$ 340.1661).

2.1.1.3 – Synthesis of the carboxylic acid 52

The acid **52** which possesses a 1,5-triazole ring, was synthesized from the aryliodide **72** in three steps (i) azidation, (ii) a ruthenium-catalysed click reaction by using $Cp^*RuCl(PPh_3)_2$ (this catalyst is known to result in the formation of a 1,5-triazole; the opposite regioisomer to that form in the CuAAC reaction)[57–59] and (iii) an ester hydrolysis (Scheme 2.4).



Scheme 2.4 – Synthesis of acid **52** from the iodo ester **72** *via* an azidation of iodo ester **72**, then Ru-catalysed [3 + 2] cycloaddition of azide **77**, followed by ester hydrolysis of **78** to achieve the target acid **52**.

The azide **77** was synthesized in 70% yield by azidation of **72** (1.00 g, 3.26 mmol) in the presence of CuI/NaN_3/ Na.ascorbate and racemic *trans-N,N'*-dimethylcyclohexane-1,2-diamine in DMSO and water (5:1) at 80 °C (Scheme 2.4). Analysis of the ^{13}C NMR spectrum revealed the resonance at δ_C 129.1 was assigned to the azide substituted carbon (C-N_3) in **77**, in contrast in the starting material **72** the analogous carbon (C-I) was assigned to a more shielded resonance at δ_C 86.5. This change in chemical shift supported the formation of the required azide **77** with additional evidence from the analysis of the FTIR spectrum and the assignment of an azide stretch to the band at ν_{max} 2116 cm^{-1}. The molecular structure of compound **77** was further confirmed by the presence of an ion peak at *m/z* 244.0715 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{10}H_{11}N_3O_3Na$ 244.0698).

The azide **77** was treated with 5-methyl-1-hexyne in the presence of $Cp^*RuCl(PPh_3)_2$[47] catalyst in 1,4-dioxane at 70 °C to achieve the triazole ester **78** in 37%

yield. Analysis of the ^{1}H NMR spectrum of **78** showed the characteristic triazole resonance as a singlet at δ_H 7.56. This chemical shift was significantly upfield of that in its regioisomer **73** which resonated at δ_H 8.16, which is consistent with the deshielding effect of the *N*-aryl substituent in **73**. The molecular structure of ester **78** was further verified by the presence of an ion peak at *m/z* 340.1638 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{17}H_{23}N_3O_3Na$ 340.1637). The target acid **52** was synthesized in 78% yield from the base catalyzed hydrolysis of the ester **78** (Scheme 2.3). The ^{1}H NMR spectrum of **52** showed the characteristic singlet resonance of the triazole proton at δ_H 7.56. The C-4 carbon of the triazole ring resonated at δ_C 131.1 and was assigned by analysis of the gHSQC spectrum which showed a correlation between this resonance and the triazole proton at δ_H 7.56 (Figure 2.7).

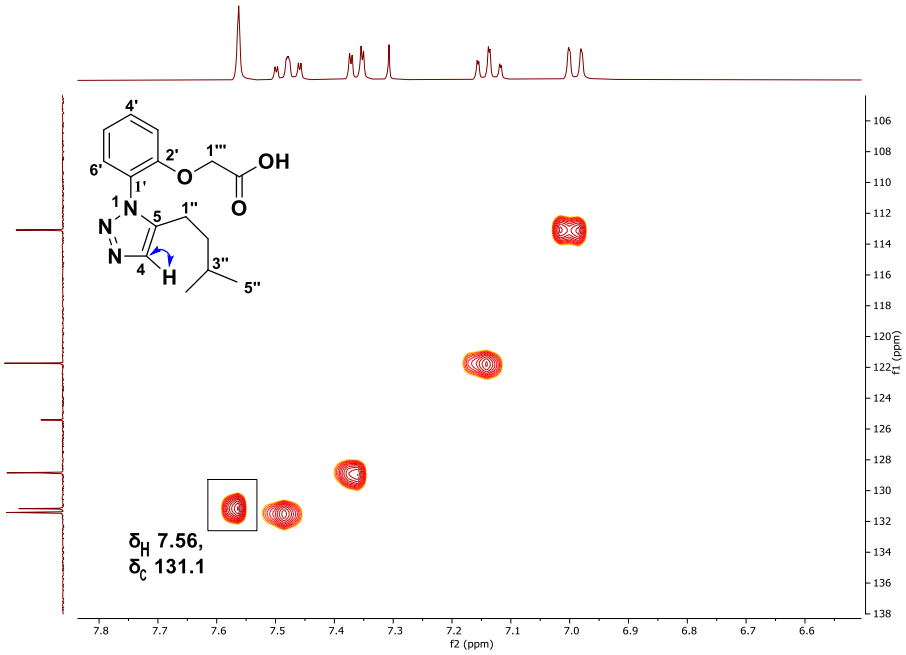


Figure 2.7: gHSQC spectrum of compound **52** (400 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-4 is highlighted.

Analysis of the gHMBC spectrum of **52** displayed a resonance at δ_C 140.8 which correlated to δ_H 7.56, this was assigned to the C-5 carbon of the triazole. Further, the resonance at δ_C 170.0 was assigned to the carbonyl carbon of the carboxylic acid as it correlated to the methylene protons (δ_H 4.57) of the carboxylic acid side chain (Figure 2.8).

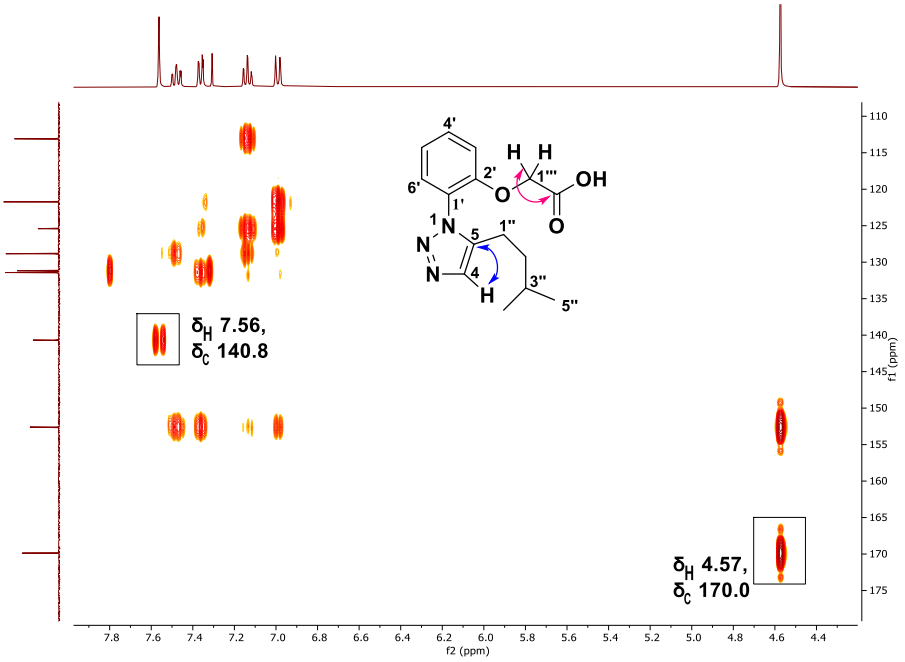


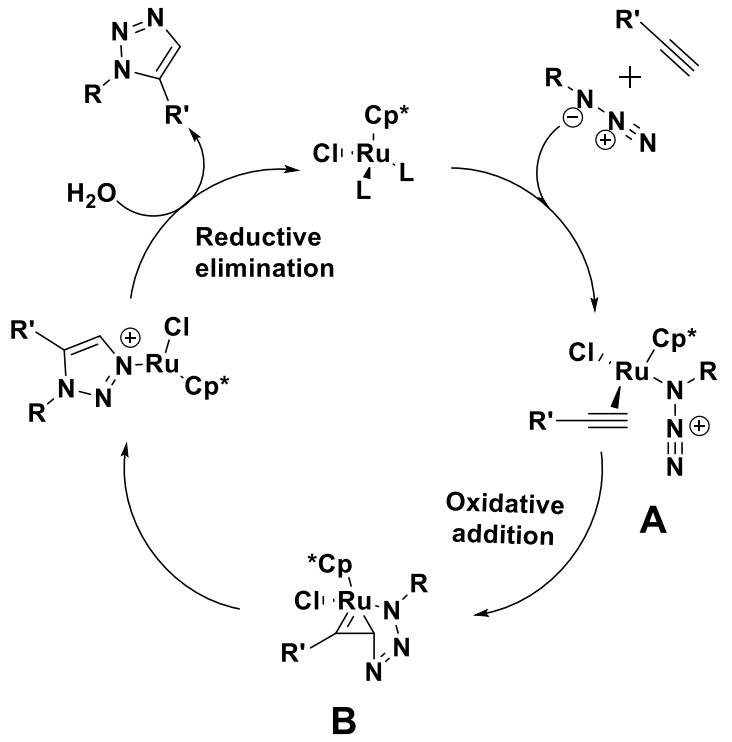
Figure 2.8: gHMBC spectrum of compound **52** (400 MHz, $CDCl_3$). The two-bond correlations between the triazole proton and C-5, H-1''' and carbonyl carbon of COOH are highlighted.

The molecular structure of acid **52** was further verified by the presence of an ion peak at *m/z* 312.1329 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{15}H_{19}N_3O_3Na$ 312.1324).

Mechanism for the Ru-catalysed cycloaddition reaction:

The Ru-catalysed click reaction requires the presence of a catalytic quantity of Ru(I); this catalyst can be added directly to the reaction. Most importantly, this allows the

reaction to be successfully conducted in non-dried solvents under an inert atmosphere. The Ru-catalyzed azide−alkyne cycloaddition (RuAAC) is proposed to occur *via* formation of the Ru-complex **A**, which undergoes an oxidative coupling process to give the ruthenacycle **B**. In this step, first the new carbon−nitrogen bond is formed between the more electron rich carbon of the alkyne and the terminal, electrophilic nitrogen of the azide (Scheme 2.5).

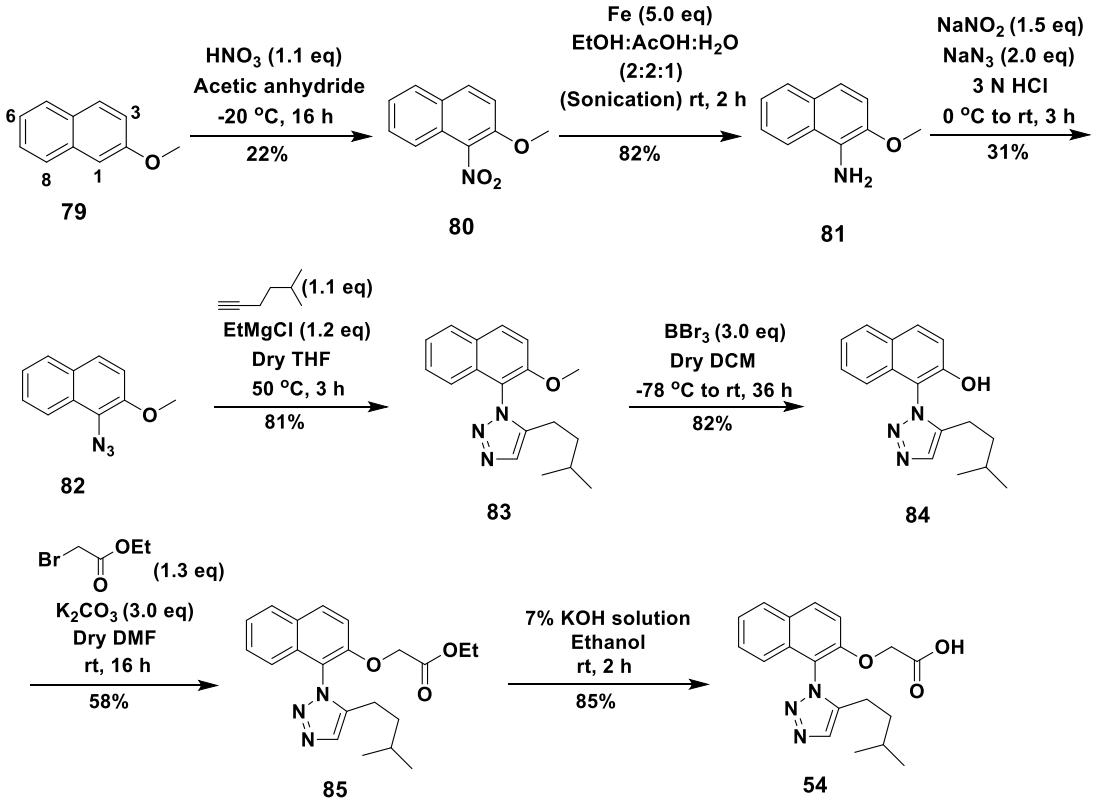


Scheme 2.5: Mechanism of Ru-catalysed cycloaddition reaction

A reductive elimination process then forms a ruthenated-triazole intermediate which upon hydrolysis gives the 1,5-disubstituted triazole product (Scheme 2.5). DFT calculations support this mechanistic proposal and indicate that the reductive elimination step is rate-determining.

2.1.1.4 – Synthesis of the carboxylic acid 54

The synthesis of acid **54** from the precursor **79** was accomplished in multiple steps involving (i) nitration, (ii) reduction, (iii) an azidation, (iv) Mg-promoted click reaction, (v) demethylation, (vi) *O*-alkylation and (vii) an ester hydrolysis (Scheme 2.6).



Scheme 2.6 – Synthesis of acid **54** from 2-methoxynaphthalene **79** *via* nitration/reduction and azidation of starting material **79**, then Mg promoted [3 + 2] cycloaddition and demethylation of azide **82**, followed by *O*-alkylation and ester hydrolysis of **84** to achieve the target acid **54**.

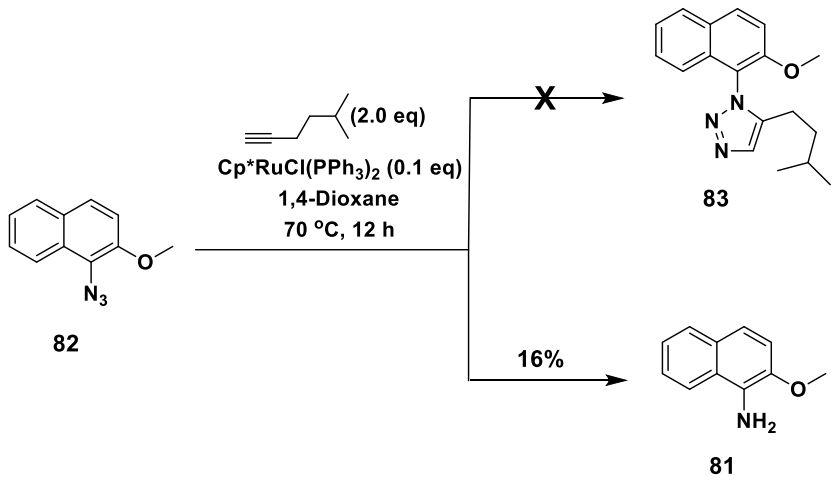
The nitro intermediate **80** was synthesized in 22% yield from 2-methoxynaphthalene **79** by nitration using HNO_3 in acetic anhydride at -20 °C (Scheme 2.6). Analysis of the ^{1}H NMR spectrum of the nitro compound **80** displayed six proton resonances in the aromatic region, whereas precursor **79** displayed seven aromatic proton resonances. Importantly the singlet resonance of precursor **79** at δ_H 7.12 assigned to H-1

was absent in the ^{1}H NMR spectrum of the nitro compound **80**. The spectroscopic data were found to be in agreement with those previously reported.[80]

Reduction of the nitro compound **80** was achieved by treatment with Fe in EtOH/AcOH/H_2O[81] under sonication conditions at rt to achieve the amine **81** in 82% yield (Scheme 2.6). Analysis of the ^{1}H NMR spectrum of amine **81** revealed a broad singlet resonance at δ_H 4.22 (2H) assigned to the -NH_2 group. The spectroscopic data were found to be in agreement with those previously reported.[81]

The azide **82** was isolated in 31% yield under Sandmeyer conditions from the amine **81** in the presence of $NaNO_2$/NaN_3 in 3 N HCl at rt (Scheme 2.6). Analysis of the ^{1}H NMR spectrum of azide **82** showed the disappearance of the broad singlet resonance at δ_H 4.22, which was assigned to the NH_2 group of **81**. Analysis of the ^{13}C NMR spectrum revealed a resonance at δ_C 122.4 assigned to the azide substituted carbon (C-N_3) of **82**, whereas the analogous carbon (C-NH_2) of **81** was assigned to a more shielded resonance at δ_C 120.2. The molecular structure of azide **82** was further confirmed by the presence of an ion peak at *m/z* 172.0789 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H - N_2]^+$) (calculated for $C_{11}H_{10}NO$ 172.0762).

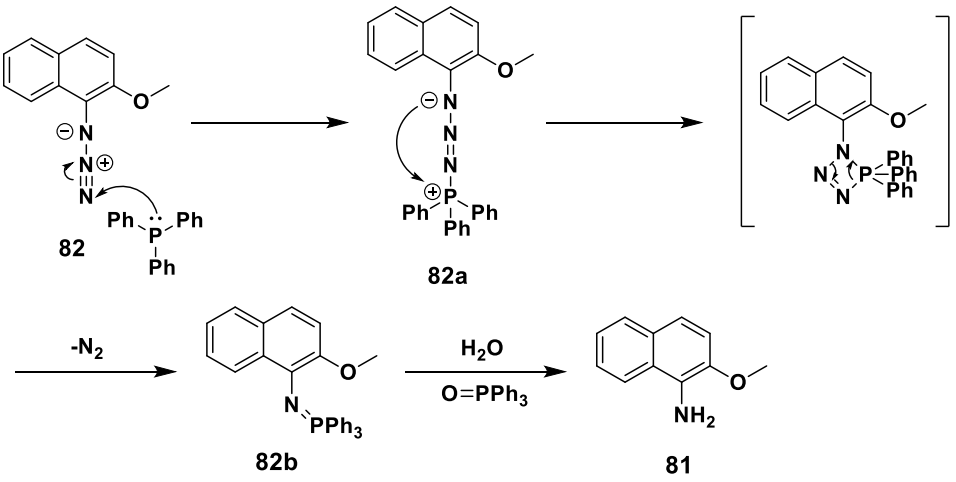
Initially the azide **82** was treated with 5-methyl-1-hexyne in the presence of Cp*RuCl(PPh_3)$_2$ in 1,4-dioxane at 70 $^{\circ}$C for 12 h. This resulted in the formation of 1-amino-2-methoxynaphthalene **81** in 16% yield, with none of the desired triazole product **83** formed. It was speculated that 0.2 equivalents of PPh_3 from the ruthenium catalyst resulted in the formation of the amine **81** *via* a Staudinger reaction on the azide group of **82** (Scheme 2.7).



Scheme 2.7– Attempted synthesis of the click product **83**

Proposed mechanism for the formation of 81:

The proposed reaction mechanism proceeds through the formation of an iminophos- phorane **82a** *via* nucleophilic addition of the triphenylphosphine to the terminal nitrogen atom of the azide and then exclusion of diatomic nitrogen to form **82b**. The intermediate **82b** undergoes hydrolysis to produce the amine **81** and triphenylphosphine oxide (Scheme 2.8).



Scheme 2.8 – Mechanism for formation of amine **81** from azide **82**

Following the failure of the Ru-catalyzed conditions to produce the 1,5-triazoles, attention turned to the Mg-promoted click reaction conditions.[92] Therefore, following conditions reported by Sharpless[92] the azide **82** was treated with chloromagnesium-5-methyl-1-hexynilide (prepared *in situ* by treating 5-methyl-1-hexyne with EtMgCl) in dry THF at 50 °C to produce the triazole **83** in 81% yield (Scheme 2.6). Analysis of the ^{1}H NMR spectrum of **83** showed the characteristic triazole singlet resonance at δ_H 7.71. The molecular structure of triazole **83** was further established by the presence of the ion peak at *m/z* 296.1750 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{18}H_{22}N_3O$ 296.1763). For further spectroscopic evidence for the formation of the 1,5-triazole regioisomer **83**, please see discussion on page 123.

The *O*-demethylation of **83** was achieved by treatment with BBr_3 in DCM at -78 °C to realize the naphthol **84** in 82% yield (Scheme 2.6). The ^{1}H NMR spectrum of naphthol **84** displayed a broad singlet resonance at δ_H 10.31, which was assigned to the OH group. The molecular structure of compound **84** was further verified by the presence of an ion peak at *m/z* 282.1602 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{17}H_{20}N_3O$ 282.1606).

The base-promoted *O*-alkylation of **84** with ethyl bromoacetate produced ester **85** in 58% yield (Scheme 2.6). Subsequent hydrolysis of **85** using 7% KOH solution in ethanol produced the target acid **54** in 85% yield (Scheme 2.6). Analysis of the ^{1}H NMR spectrum of **54** revealed the characteristic triazole proton resonance at δ_H 7.74 (s, 1H). The C-4 carbon of the triazole ring resonated at δ_C 131.4 and was assigned from the

gHSQC correlation of the triazole proton (δ_H 7.74) to the resonance at δ_C 131.4 (Figure 2.9).

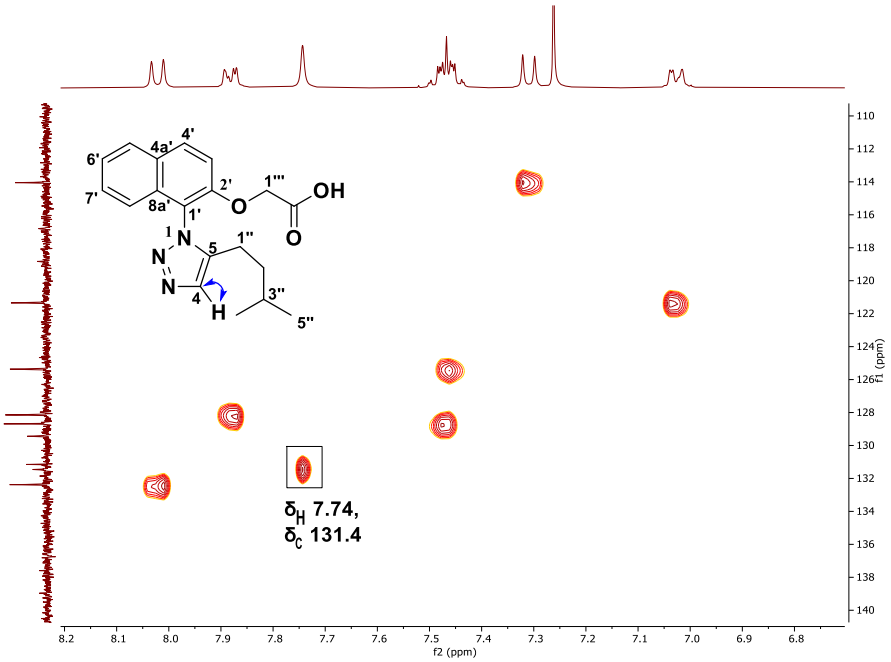


Figure 2.9: gHSQC spectrum of compound **54** (400 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-4 is highlighted.

The gHMBC spectrum allowed the assignment of the resonance δ_C 141.4 to the quaternary C-5 carbon of the triazole (Figure 2.10), while the resonance of the carbonyl carbon of the carboxylic acid at δ_C 170.0 correlated to the methylene protons (δ_H 4.72) of the acid side chain (Figure 2.10).

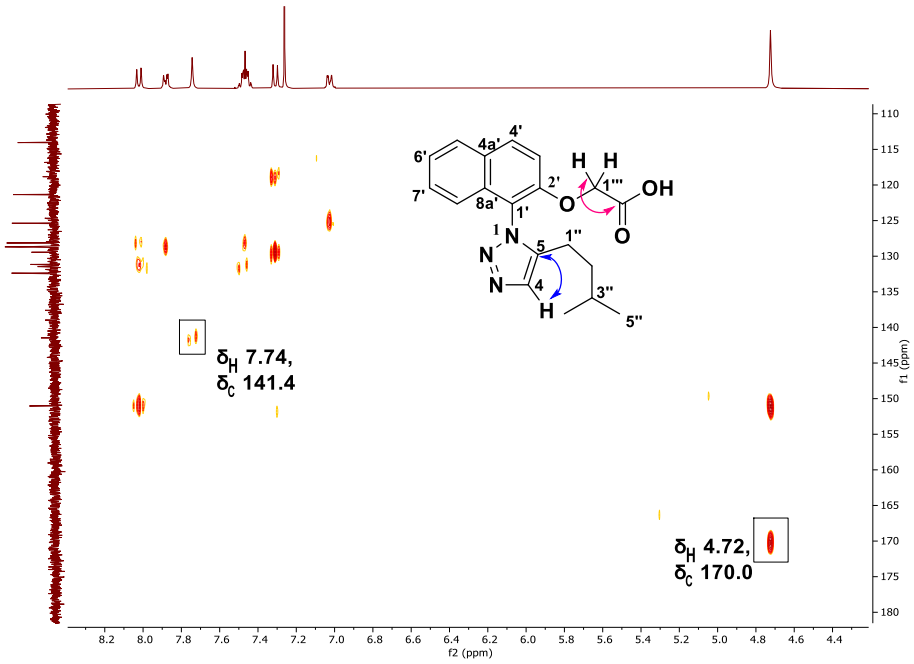
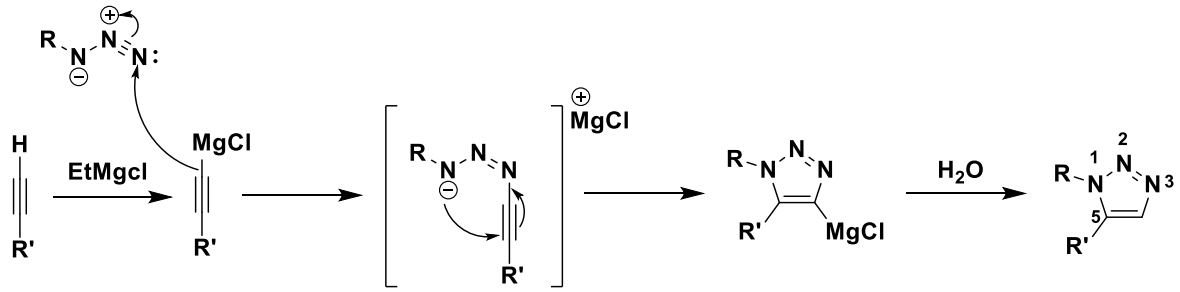


Figure 2.10: gHMBC spectrum of compound **54** (400 MHz, $CDCl_3$). The two-bond correlations between the triazole proton and C-5, H-1''' and carbonyl carbon of COOH are highlighted.

The molecular structure of compound **54** was further confirmed by the presence of an ion peak at *m/z* 340.1666 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{19}H_{22}N_3O_3$ 340.1661).

Mechanism for the Mg-promoted cycloaddition reaction:

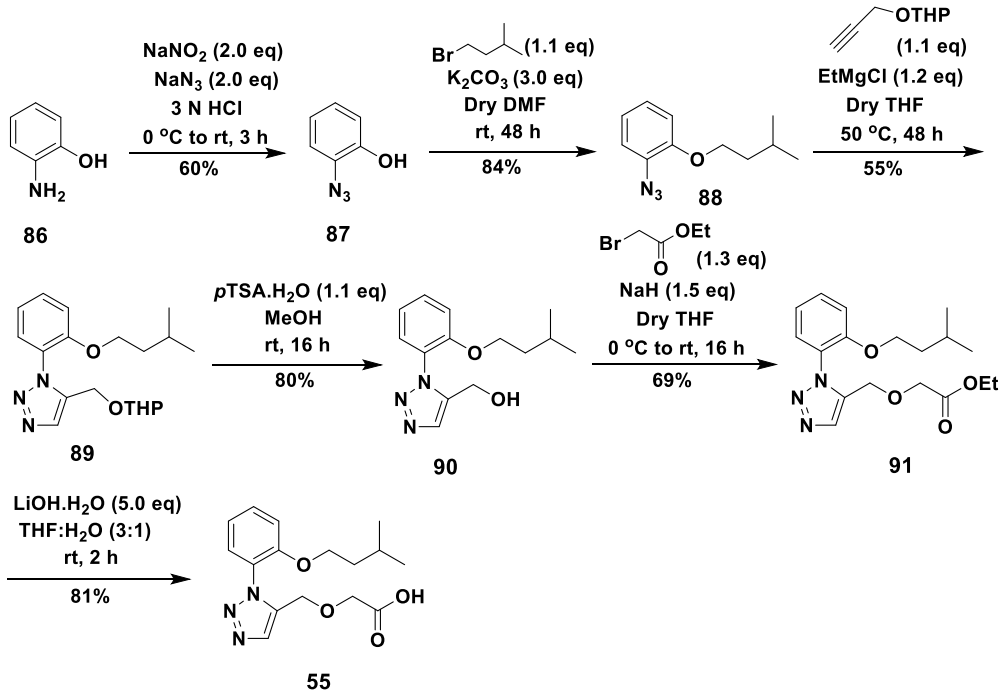
The accepted mechanism (Scheme 2.9) starts with the formation of chloromagnesium acetylide by treatment of the alkyne with EtMgCl. Then nucleophilic addition of the acetylide to the terminal nitrogen atom of the azide occurs, followed by spontaneous five membered ring closure to the chloromagnesium-4-metallotriazole species. Hydrolysis of this species then gives the 1,5-disubstituted triazole product, which is regioisomeric with the product formed using the Cu-catalysed azide-alkyne cycloaddition (CuAAC) reaction procedure.



Scheme 2.9: Proposed mechanism of Mg-promoted cycloaddition reaction

2.1.1.5 – Synthesis of the carboxylic acid 55

The synthesis of acid **55** from precursor **86** was accomplished in multiple steps involving (i) azidation, (ii) an *O*-alkylation, (iii) Mg-promoted click reaction, (iv) THP deprotection, (v) an *O*-alkylation and (vi) an ester hydrolysis (Scheme 2.10).



Scheme 2.10 – Synthesis of acid **55** from 2-aminophenol **86** *via* an azidation, *O*-alkylation and Mg promoted [3 + 2] cycloaddition of **86**, then THP deprotection and *O*-alkylation of **89**, followed by ester hydrolysis of **91** to furnish target acid **55**.

The azide **87** was isolated in 60% yield following a Sandmeyer reaction on 2-aminophenol **86** in the presence of $NaNO_2/NaN_3$ in 3 N HCl at rt (Scheme 2.10). The spectroscopic data of **87** were found to be in agreement with those previously reported.[79]

The base-promoted *O*-alkylation of azide **87** with 1-bromo-3-methylbutane was achieved with K_2CO_3 in dry DMF at rt (Scheme 2.10) and produced the ether **88** in 84% yield. The ether **88** was treated with the chloromagnesium acetylide formed from the reaction of tetrahydro-2-(2-propynyloxy)-2*H*-pyran (1.1 eq) with EtMgCl in dry THF at 50 °C to achieve the triazole **89** in 55% yield (Scheme 2.10). The ^{1}H NMR spectrum of **89** displayed the characteristic resonance of the triazole ring proton at δ_H 7.76 (s, 1H). The molecular structure of compound **89** was further established by the presence of an ion peak at *m/z* 368.1964 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{19}H_{27}N_3O_3Na$ 368.1950).

The THP deprotection of **89** was achieved using *p*-toluenesulfonic acid. monohydrate in MeOH at rt to obtain the alcohol **90** in 80% yield (Scheme 2.10). Analysis of the ^{1}H NMR spectrum of **90** showed a characteristic broad singlet resonance at δ_H 3.95, which was assigned to the hydroxy group of the newly formed alcohol **90**. The molecular structure of compound **90** was further verified by the presence of an ion peak at *m/z* 284.1393 in the HRMS that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{14}H_{19}N_3O_2Na$ 284.1375).

The base-promoted an *O*-alkylation of **90** with ethyl bromoacetate produced ester **91** in 69% yield (Scheme 2.10). The desired carboxylic acid **55** was realized in 81% yield by the base hydrolysis of **91** (Scheme 2.10). The ^{1}H NMR spectrum of **55** showed the

characteristic singlet resonance for the triazole proton at δ_H 7.88 which was correlated in the gHSQC spectrum to the resonance at δ_C 132.7 which was assigned to C-4 (Figure 2.11).

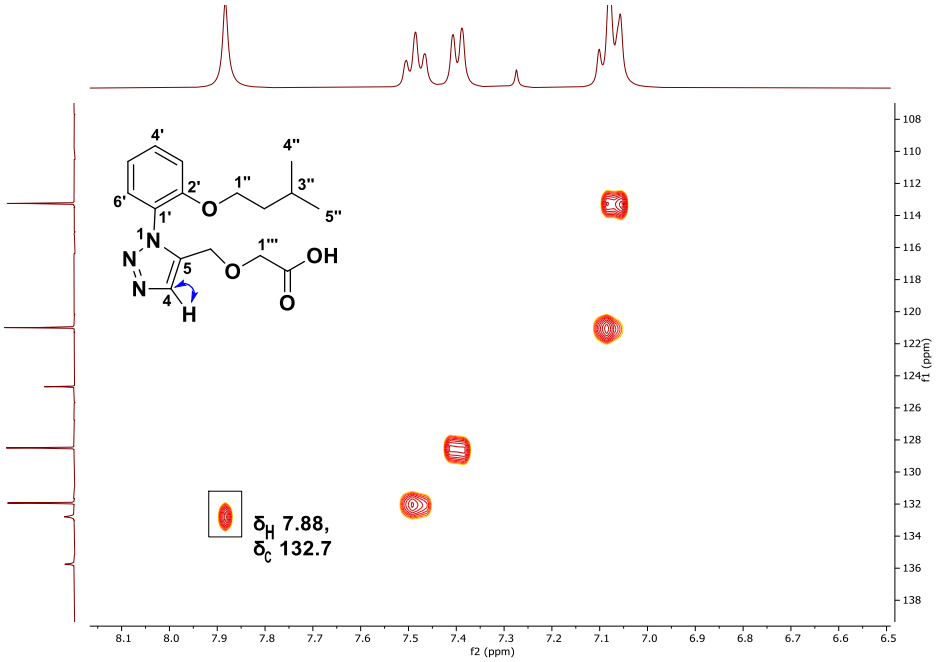


Figure 2.11: gHSQC spectrum of compound **55** (400 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-4 is highlighted.

In the gHMBC spectrum, the resonance at δ_H 7.88 correlated to the resonance at δ_C 135.7 which was assigned to the quaternary C-5 carbon (Figure 2.12), while the resonance at δ_C 173.1 was assigned to the carbonyl carbon of the carboxylic acid as it correlated to the methylene protons that resonated at δ_H 4.01 (Figure 2.12).

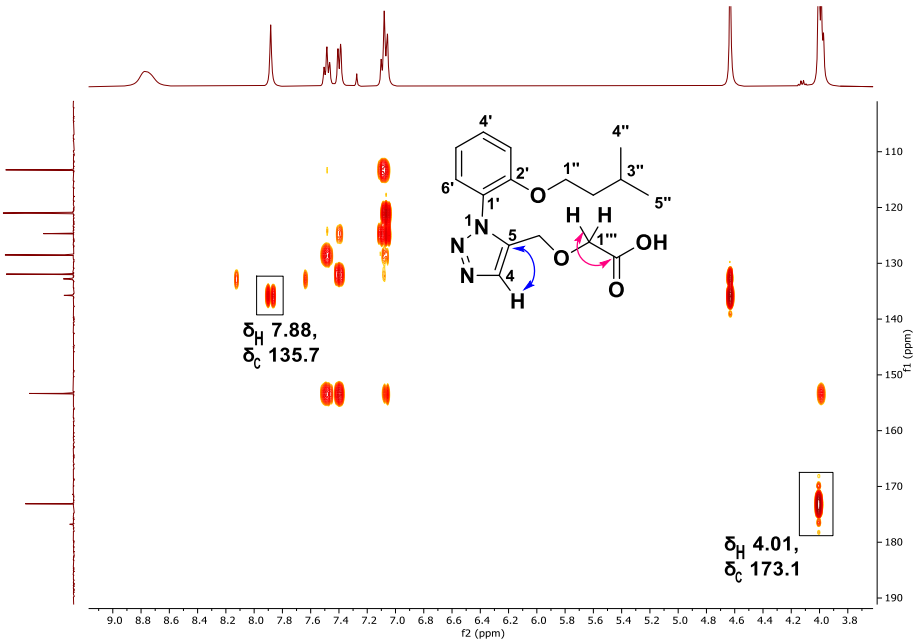


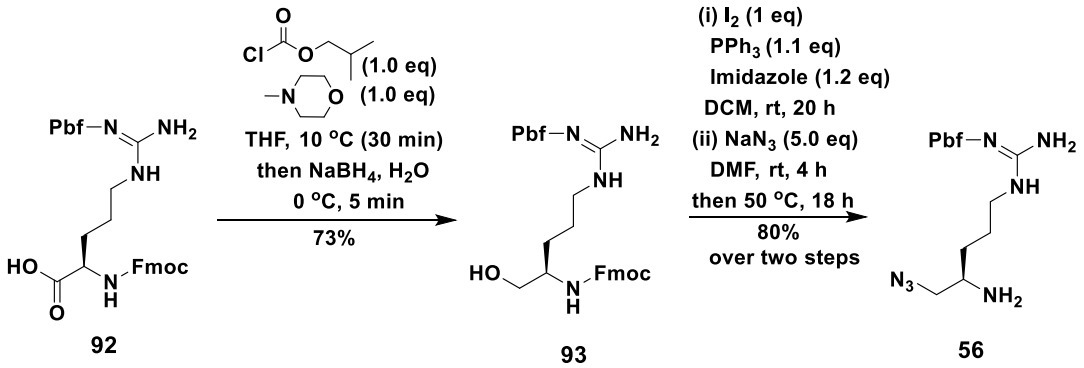
Figure 2.12: gHMBC spectrum of compound **55** (400 MHz, $CDCl_3$). The two-bond correlations between the triazole proton and C-5, H-1''' and carbonyl carbon of COOH are highlighted.

The molecular structure of acid **55** was further confirmed by the presence of an ion peak at *m/z* 320.1603 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{16}H_{22}N_3O_4$ 320.1610).

2.1.2 – Synthesis of the *N*-protected β-azidoamines

2.1.2.1 – Synthesis of the *N*-protected β-azidoamine 56

The synthesis of β-azidoamine **56** from commercially available Fmoc-(D)-Arg(Pbf)-OH **92** was achieved by carboxylic acid reduction, iodination and subsequent azidation/*N*-deprotection (Scheme 2.11) as previously described.[47]



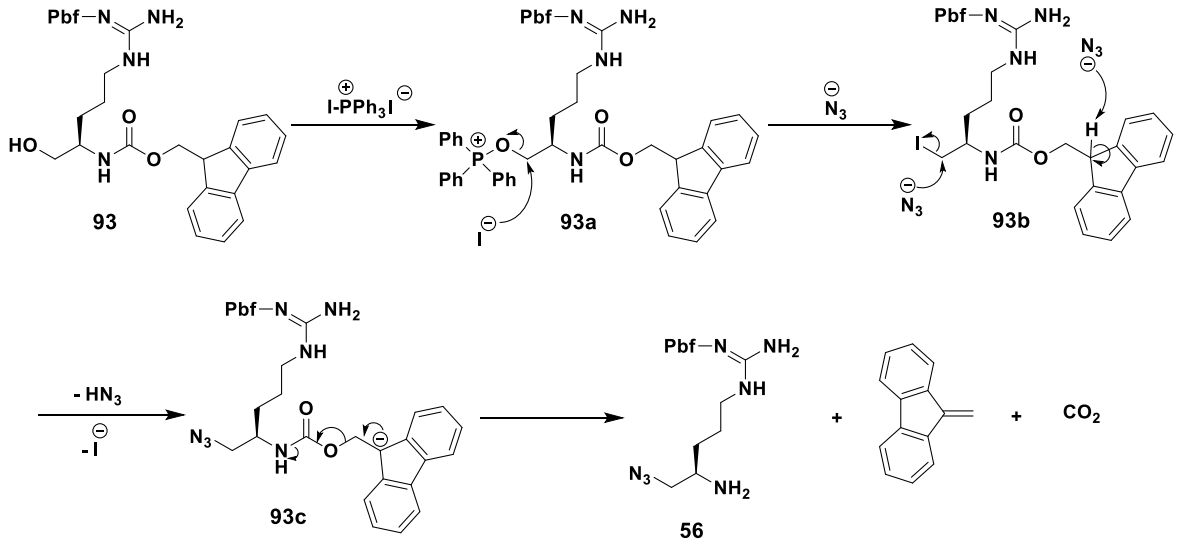
Scheme 2.11 – Synthesis of the azide **56** from the arginine precursor **92** *via* reduction of the carboxylic acid **92** to give alcohol **93**, followed by iodination and then azidation/*N*-deprotection to furnish target azide **56.**

Reduction of the precursor acid **92** was achieved *via* the mixed anhydride formed by the treatment of the acid **92** with isobutyl chloroformate and *N*-methylmorpholine which upon $NaBH_4$ reduction gave the alcohol **93** in 73% yield. The alcohol was iodinated with I_2/PPh_3/imidazole in CH_2Cl_2; this step was followed by an azidation reaction at rt and a *N*-Fmoc deprotection at 50 °C with NaN_3 in DMF to give the target β-azidoamine **56** in 80% yield over the two steps (Scheme 2.11). Compound **56** exhibited spectroscopic data that were in agreement with those reported previously.[47]

Mechanism for the formation of 56:

The reaction mechanism starts with the formation of the iodo phosphonium salt which reacts with the alcohol **93** to give the triphenyloxyphosphonium salt **93a**. The nucleophilic displacement of this intermediate **93a** by an iodide anion produces the iodide **93b** (Scheme 2.12) and triphenylphosphine oxide. Further nucleophilic displacement of the intermediate **93b** by azide anion produces the corresponding azide which at the higher temperature of 50 °C, undergoes deprotonation highly acidic methine

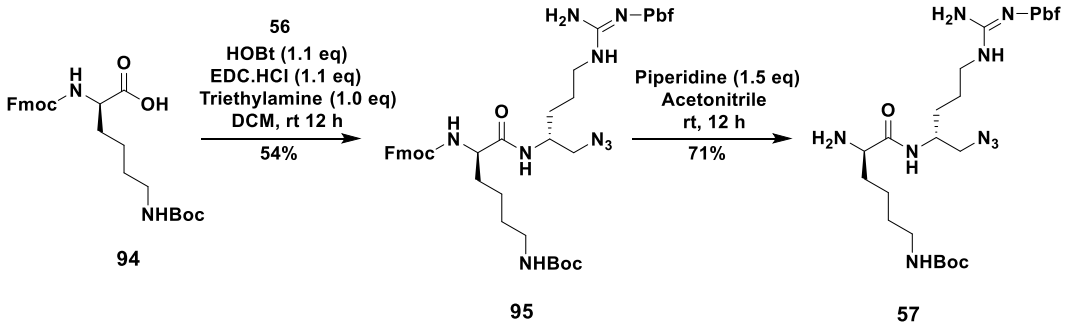
proton (on the β-carbon) of the Fmoc group by azide to give the resonance stabilized 14π – aromatic anion **93c**. This stabilized anion **93c** then triggers the departure of CO_2 and dibenzofulvene to produce the β-azidoamine **56** (Scheme 2.12).



Scheme 2.12 – Mechanism for the β-azido-amine **56** formation

2.1.2.2 – Synthesis of *N*-protected β-azidoamine 57

The synthesis of doubly *N*-protected β-azidoamine **57** was achieved *via* a peptide coupling reaction of β-azidoamine **56** with commercially available Fmoc-L-Lys(Boc)-OH **94**, followed by Fmoc-deprotection (Scheme 2.13) using piperidine.



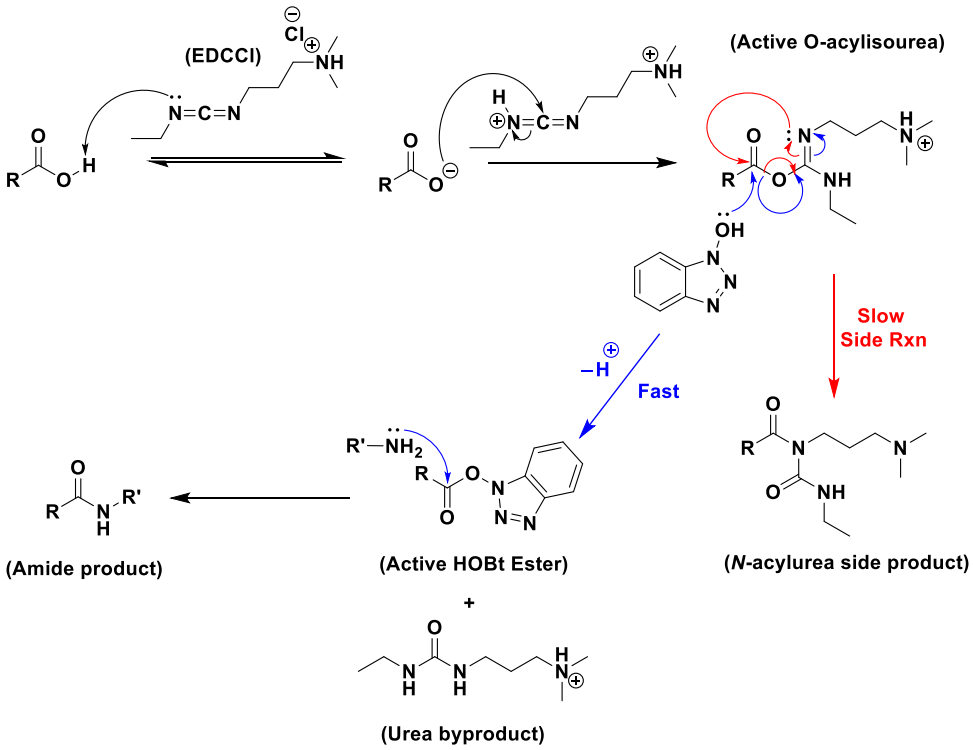
Scheme 2.13 – Synthesis of azide **57** from amine **56** *via* peptide coupling of amine **56** with Fmoc-L-Lys(Boc)-OH **94** to give compound **95**, followed by Fmoc deprotection of **95** to furnish target azide **57**

The peptide coupling of β-azidoamine **56** with Fmoc-L-Lys(Boc)-OH **94** in the presence of HOBt/EDC.HCl and triethylamine in DCM at rt gave product **95** in 54% yield. The intermediate **95** was then subjected to deprotection with piperidine in MeOH at rt to give the target azide **57** in 71% yield. Furthermore, a traditional acid/base work-up procedure was utilized to purify the β-azidoamine **57** product instead of flash chromatography, which led to a substantially increased overall yield from the literature value of 64 % to 71% and a significantly easier isolation procedure (Scheme 2.13). Compound **57** exhibited spectroscopic data that were in agreement with those reported previously.[47]

Mechanism of peptide coupling:

Amide bonds are typically constructed from the union of carboxylic acids and amines. However, the unification of these two functional groups does not occur spontaneously at ambient temperature with the necessary elimination of water only taking place at high temperatures (e.g. >200 °C). The higher temperature conditions are typically detrimental to the integrity of the highly functionalized acids and amines. For this reason,

peptide coupling reagents such as HOBt (1-hydroxy-1*H*-benzotriazole) and EDCI [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride)] are necessary for amide bond formation under mild reaction conditions.[70-71] Initially the EDCI activates the carboxylic acid as an *O*-acylisourea intermediate by the reaction of the carboxylate anion with EDCI. Then the *O*-acylisourea reacts with the precursor amine to produce the required amide product (Scheme 2.14). A side reaction is also possible by the activated *O*-acylisourea undertaking an intramolecular acyl transfer to provide an inactive *N*-acylurea by-product (Scheme 2.14 – red route),[70-72] that consumes the acid without producing the desired product amide.



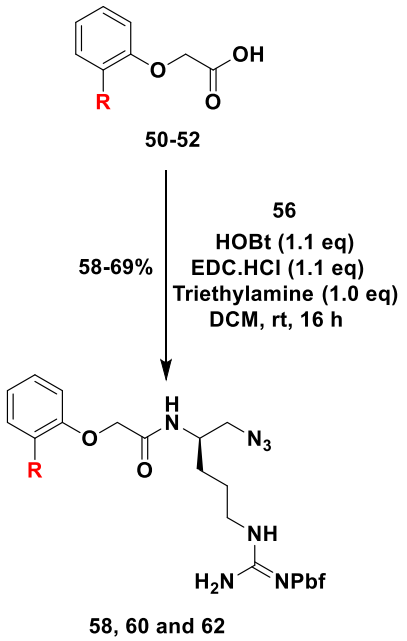
Scheme 2.14 – Mechanism of EDCI/HOBt promoted amide coupling: both the product pathway (blue) and the undesired side reaction (red) are displayed

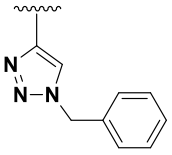
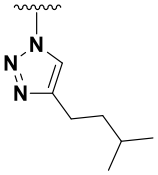
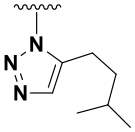
The nucleophilic additive HOBt is added to prevent this side reaction.[70-72] The active *O*-acylisourea reacts with HOBt to produce an HOBt ester (Scheme 2.14 – blue route) that quickly undergoes aminolysis by reacting with the precursor amine to give the desired product amide.[70-71]

2.2 – Synthesis of the *N*-protected azides 1 – 11

2.2.1 – Synthesis of the azides 58, 60 and 62

The target azides **58**, **60** and **62** were achieved in 58 – 69% yield from the coupling reaction of the acids **50 – 52** and the β-azidoamine **56** in the presence of HOBt/EDC.HCl and triethylamine in DCM at rt for 16 h (Scheme – 2.15).



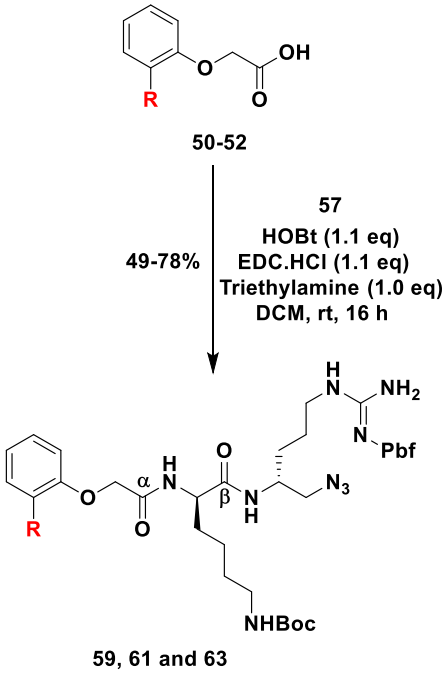
Acid	Azide	R	Yield (%)
50	58		69
51	60		58
52	62		65

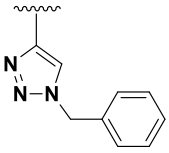
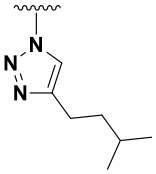
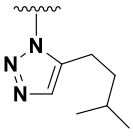
Scheme 2.15 – Synthesis of the azides **58**, **60** and **62** from the acids **50 – 52** *via* peptide coupling

The ^{1}H NMR spectrum of the azide **58** displayed a broad singlet resonance at δ_H 7.51 for the CON*H* group and the resonance at δ_C 169.6 was assigned to the carbonyl carbon of the new amide group based on the gHMBC correlation of this resonance with that of the NH proton. The molecular structure of compound **58** was verified by the presence of an ion peak at *m/z* 751.3108 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{36}H_{44}N_{10}O_5SNa$ 751.3109). The other azides **60** and **62** in Scheme 2.15, was also fully characterized by NMR and HRMS analysis.

2.2.2 – Synthesis of the azides 59, 61 and 63

The azides **59**, **61** and **63** were isolated in 49-78% yield from the coupling reaction of the acids **50** – **52** with the β-azidoamine **57** (Scheme – 2.16).



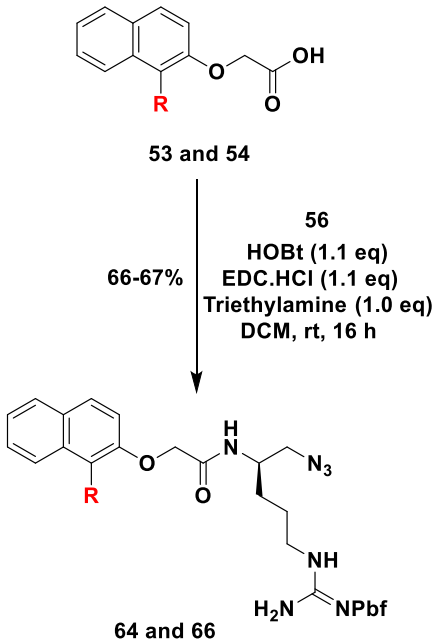
Acid	Azide	R	Yield (%)
50	59		49
51	61		78
52	63		74

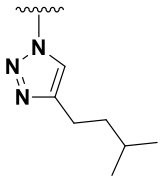
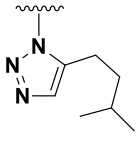
Scheme 2.16 – Synthesis of the azides **59**, **61** and **63** from the acids **50 – 52** *via* peptide coupling

The structure of **59** was evident from the broad singlet resonance at δ_H 8.98 for the α-CON*H* group and the resonance at δ_C 165.5 was assigned to the carbonyl carbon of the new amide group based on the gHMBC correlation of this resonance with that of the NH proton. The molecular structure of compound **59** was further confirmed by the presence of an ion peak at *m/z* 979.4587 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{47}H_{64}N_{12}O_8SNa$ 979.4583). The other azides **61** and **63** in Scheme 2.16, were also fully characterized by NMR and HRMS analysis.

2.2.3 – Synthesis of the azides 64 and 66

The required azides **64** and **66** were obtained in yields 66 and 67%, respectively from the coupling reaction of the acid **53** and **54** with the β-azidoamine **56** (Scheme – 2.17).



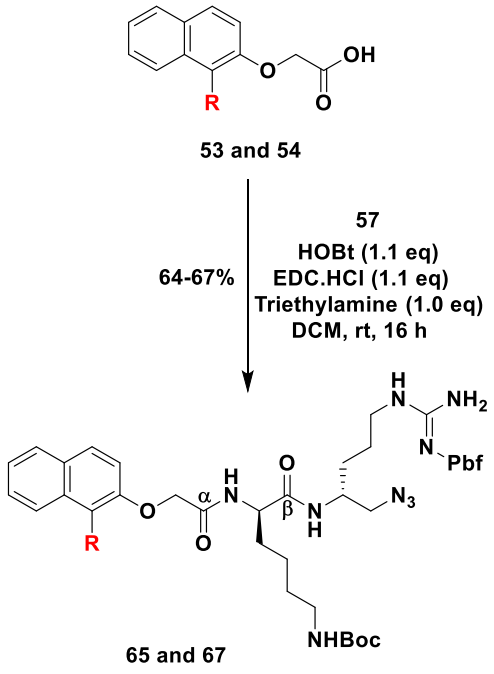
Acid	Azide	R	Yield (%)
53	64		67
54	66		66

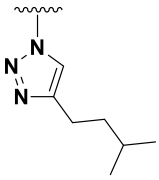
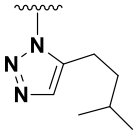
Scheme 2.17 – Synthesis of the azides **64** and **66** from the acids **53** and **54** *via* peptide coupling

In the ^{1}H NMR spectrum of the azide **64** showed a broad singlet resonance at δ_H 6.66 which was assigned to the CON*H* group and the resonance at δ_C 167.5 was assigned to the carbonyl carbon of the new amide group based on their gHMBC correlations. The molecular structure of compound **64** was further established by the presence of an ion peak at *m/z* 781.3620 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{38}H_{50}N_{10}O_5SNa$ 781.3584). The other azide **66** in Scheme 2.17, was also fully characterized by NMR and HRMS analysis.

2.2.4 – Synthesis of the azides 65 and 67

The coupling reaction of the acids **53** and **54** with the β-azidoamine **57** resulted in yields 64 and 67%, respectively of the azides **65** and **67** (Scheme – 2.18).



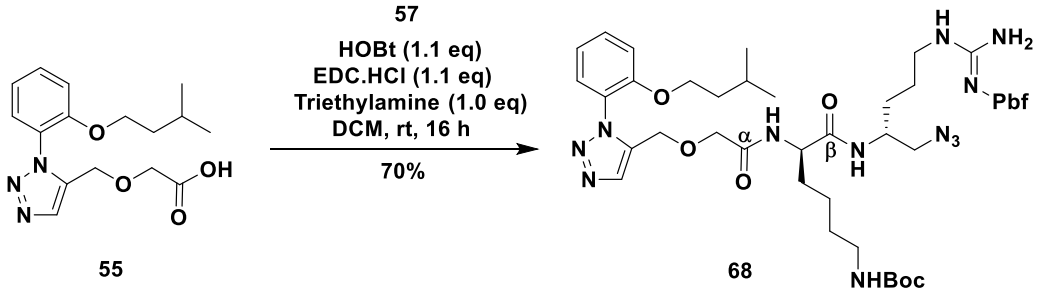
Acid	Azide	R	Yield (%)
53	65		64
54	67		67

Scheme 2.18 – Synthesis of the azides **65** and **67** from the acids **53** and **54** *via* peptide coupling

The structure of **65** was evident from the broad singlet resonance at δ_H 6.85 for the α-CON*H* group. The ^{13}C NMR spectrum showed a resonance at δ_C 168.1 which was assigned to the carbonyl carbon of the new amide group. The molecular structure of compound **65** was verified by the presence of an ion peak at *m/z* 987.5272 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{49}H_{71}N_{12}O_8S$ 987.5239). The other azide **67** in Scheme 2.18, was also fully characterized by NMR and HRMS analysis

2.2.5 – Synthesis of the azide 68

The azide **68** was isolated in 70% yield from the coupling reaction of the acid **55** with the β-azidoamine **57** (Scheme – 2.19).



Scheme 2.19 – Synthesis of the azide **68** from the acid **55** *via* peptide coupling

The ^1H NMR spectrum of **68** displayed a broad singlet resonance at δ_H 6.97 for the α-CON*H* group. The ^{13}C NMR spectrum showed a resonance of the new amide carbonyl group at δ_C 169.1 was assigned based on their gHMBC correlations. The molecular structure of compound **68** was verified by the presence of an ion peak at *m/z* 989.5029 in the HRMS (ESI) that was assigned to the sodiated molecular ion ($[M + Na]^+$) (calculated for $C_{46}H_{70}N_{12}O_9SNa$ 989.5007).

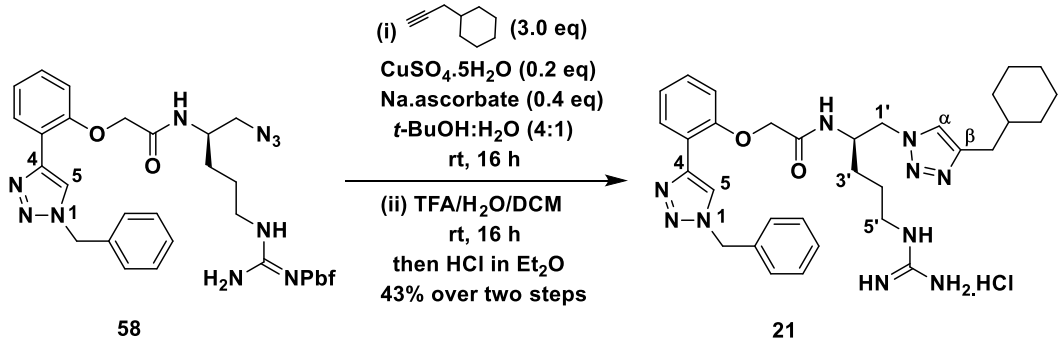
2.3 – Synthesis of derivatives

2.3.1 – Synthesis of the monocationic derivatives

The derivatization of the monocationic amino acid derivatives **21 – 34** required a two-step transformation – installation of the terminal triazole moieties by using the azides **58**, **60**, **62**, **64**, **66** and alkynes *via* a CuAAC reaction, followed by *N*-Pbf group cleavage with CH_2Cl_2/H_2O/TFA.

Synthesis of the derivative 21 from the azide 58:

The hydrochloride salt derivative of **21** was synthesized in 43% yield over the two steps from the reaction of azide **58** with 3-cyclohexyl-1-propyne, $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O at rt for 16 h (Scheme 2.20). The *N*-protected triazole derivative so obtained was then subjected to *N*-Pbf deprotection with TFA and 20 equivalents of H_2O in CH_2Cl_2 at rt for 16 h followed by the treatment of the resulting TFA salt with ethereal HCl and purification by precipitation.



Scheme 2.20 – Derivatization of the azide **58** *via* installation of the terminal triazole moiety followed by deprotection of the *N*-Pbf side chain protecting group to give derivative **21** as hydrochloride salt

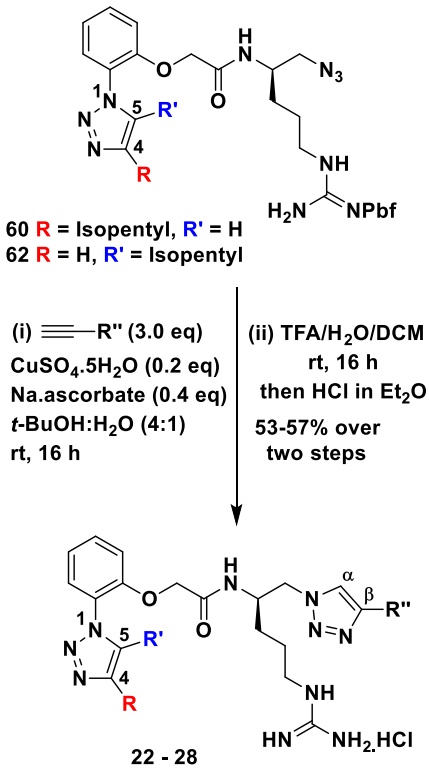
The ^{1}H NMR spectrum of derivative **21** displayed characteristic resonances that were assigned to the terminal cyclohexylmethyl group (i.e., a multiplet resonance at δ_H

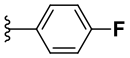
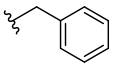
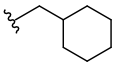
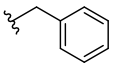
2.36 – 2.24 (2H; C<u>H</u>$_2$Cy) and cyclohexyl multiplet resonances at δ_H 1.84 – 1.40, δ_H 1.16 – 1.02 and δ_H 0.88 – 0.78). Importantly, the ^{1}H NMR spectrum of **21** showed two singlet resonances that were assigned to the two triazole ring protons at δ_H 8.59 (H5) and δ_H 7.98 (Hα). These proton resonances correlated to the carbon resonances at δ_C 131.2 (C-5) and δ_C 125.4 (C-α), respectively. The carbon resonances at δ_C 145.8 (C-4) and δ_C 131.3 (C-β) were assigned to the quaternary carbons of the triazole rings based on the gHMBC correlations of these carbon resonances to those of their triazole ring protons [δ_H 8.59 (H5) and δ_H 7.98 (Hα)], respectively. The arginine peptide chain was confirmed from the gHMBC correlations of H-1' proton (δ_H 4.74) with carbon resonances C-2' (δ_C 50.7), C-3' (δ_C 29.9) and C-α (δ_C 125.4), respectively. The H-2' proton (δ_H 4.48-4.42) correlated with carbon resonances C-1' (δ_C 56.2), C-3' (δ_C 29.9) and C-4' (δ_C 26.4). Furthermore, H-5' proton (δ_H 3.22-3.12) correlated with carbon resonances C-3' (δ_C 29.9), C-4' (δ_C 26.4) and guanidine C=N (δ_C 158.7). The successful *N*-Pbf deprotection was clearly seen by the lack of five characteristic Pbf singlet methyl resonances in the ^{1}H NMR spectrum of derivative **21**. The molecular structure of the derivative **21** was further confirmed by the appearance of the ion peak at *m*/*z* 599.3562 in the HRMS (ESI) that was assigned to the protonated molecular ion $[M - HCl + H]^+$ (calculated for $C_{32}H_{43}N_{10}O_2$ 599.3565).

Synthesis of the derivatives 22 – 28 from the azides 60 and 62:

The derivatives **22** – **28** (Scheme 2.21) were realized in yields ranging from 53 – 57% over the two steps from the azides **60** and **62** by reaction with their respective alkynes, $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O at rt for 16 h. The *N*-protected triazole

derivatives so obtained were then subjected to *N*-Pbf deprotection with TFA and 20 equivalents of H_2O in CH_2Cl_2 at rt for 16 h followed by the treatment with ethereal HCl and purification of the HCl salts by precipitation.



Compound	R	R'	R''	Yield (%)[a]
22	Isopentyl	H		55
23	Isopentyl	H		57
24	Isopentyl	H		54
25	Isopentyl	H		53
26	H	Isopentyl		56
27	H	Isopentyl		54
28	H	Isopentyl		55

[a] Yields are reported over two steps from 60 and 62

Scheme 2.21 – Derivatization of the azides **60** and **62** *via* installation of the terminal triazole moiety followed by deprotection of the *N*-Pbf side chain protecting group to give derivatives **22** – **28** as hydrochloride salts

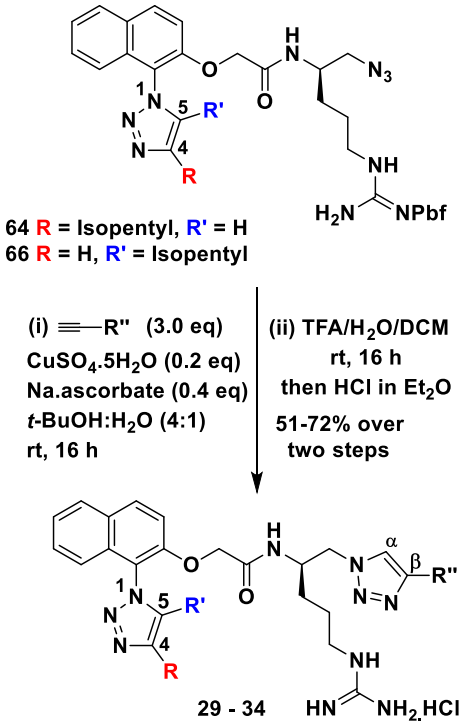
The ^{1}H NMR spectrum of the derivative **22** showed characteristic resonances (11H) that were assigned to the cyclohexyl group at δ_H 1.86 – 1.50 (m, 6H) and δ_H 1.48 – 1.20 (m, 5H). Importantly, the gHSQC spectrum of **22** showed that the two singlet resonances assigned to the two triazole ring protons at δ_H 8.46 (H5) and δ_H 8.36 (Hα) were correlated to the carbon resonances at δ_C 125.9 (C-5) and δ_C 125.3 (C-α), respectively. The ^{13}C NMR spectrum showed the resonances at δ_C 147.9 (C-β) and δ_C 132.8 (C-4) were assigned to the quaternary carbons of the triazoles based on the gHMBC correlations of

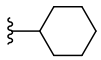
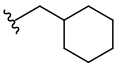
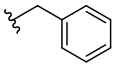
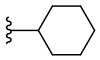
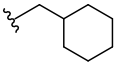
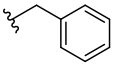
these resonances to those of their triazole ring protons [δ_H 8.46 (H5) and δ_H 8.36 (Hα)], respectively. The deprotection of *N*-Pbf was clearly seen by the lack of five characteristic Pbf singlet methyl resonances in the ^{1}H NMR spectrum of **22**. The molecular structure of the derivative **22** was established by the appearance of the ion peak at *m*/*z* 565.3731 in HRMS (ESI) that was assigned to the protonated molecular ion $[M - HCl + H]^+$ (calculated for $C_{29}H_{45}N_{10}O_2$ 565.3727). The other derivatives **23** – **28** in Scheme 2.21, were also fully characterized by NMR and HRMS analysis.

Synthesis of the derivatives 29 – 34 from the azides 64 and 66:

The derivatives **29** – **34** (Scheme 2.22) were prepared in yields ranging from 51 – 72% over the two steps from the azides **64** and **66** by reaction with their respective alkynes, $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O at rt for 16 h. The *N*-protected triazole derivatives so obtained were then subjected to *N*-Pbf deprotection with TFA and 20 equivalents of H_2O in CH_2Cl_2 at rt for 16 h followed by the treatment with ethereal HCl and purification by precipitation.

The ^{1}H NMR spectrum of the derivative **29** displayed characteristic resonances (11H) for the cyclohexyl group, a singlet resonance at δ_H 2.72 (1H), and multiplet resonances at δ_H 1.79 – 1.57 (5H) and δ_H 1.20 – 1.16 (5H). The gHSQC spectrum of **29** showed that the two singlet resonances assigned to the two triazole ring protons at δ_H 8.36 (H5) and δ_H 8.18 (Hα) were correlated to the carbon resonances at δ_C 125.7 (C-5) and δ_C 125.2 (C-α), respectively. The gHMBC spectrum allowed the assignment of the quaternary carbons of the triazole rings at δ_C 149.3 (C-4) and δ_C 147.6 (C-β).



Compound	R	R'	R''	Yield (%)[a]
29	Isopentyl	H		72
30	Isopentyl	H		66
31	Isopentyl	H		68
32	H	Isopentyl		52
33	H	Isopentyl		51
34	H	Isopentyl		57

[a] Yields are reported over two steps from 64 and 66

Scheme 2.22 – Derivatization of the azides **64** and **66** *via* installation of the terminal triazole moiety followed by deprotection of the *N*-Pbf side chain protecting group to give derivatives **29 – 34** as hydrochloride salts

The molecular structure of the derivative **29** was further verified by the appearance of the ion peak at *m*/*z* 615.3877 in HRMS (ESI) that was assigned to the protonated molecular ion, $[M - HCl + H]^+$ (calcd for $C_{33}H_{47}N_{10}O_2$ 615.3886). The other derivatives **30** – **34** in Scheme 2.22, were also fully characterized by NMR and HRMS analysis.

The derivatives **32 – 34** displayed amide rotamers (i.e. *anti* and *syn*) which were evident by the presence of smaller, additional resonances in their ^1H and ^{13}C NMR spectra.

Rotamers:

Single C–N bonds that are connected to an adjacent carbon–heteroatom double bond (e.g. C=O or C=N) are well-known to display partial double bond characteristic due

to the delocalization of the nitrogen's lone pair of electrons; this leads to restricted rotation of the C–N bond. If the energy barrier to rotation is high, two distinct rotameric conformations will be generated (i.e. *anti*- and *syn*-rotamers – Figure 2.13), with the *anti*-rotamer generally being preferred in secondary amides due to steric considerations.[61]

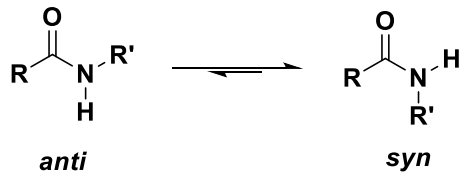


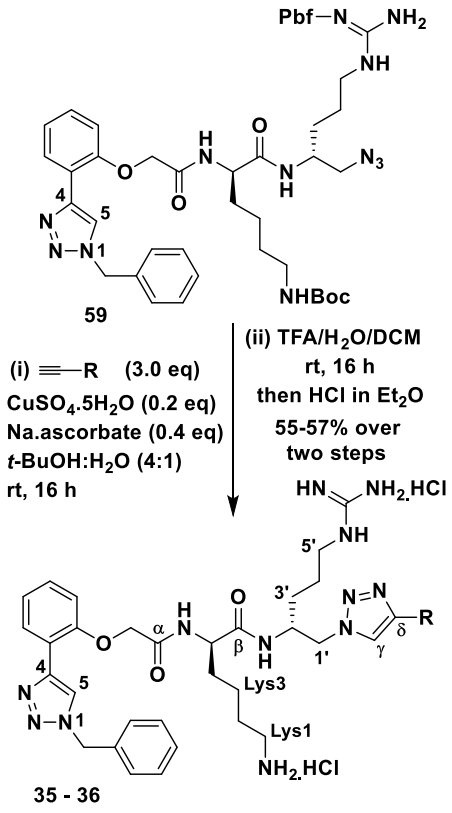
Figure 2.13 – Generic *anti*- and *syn*-rotamers

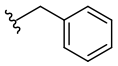
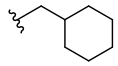
2.3.2 – Synthesis of the dicationic derivatives

The derivatization of the dual-amino acid derivatives **35 – 49** required a two-step transformation – installation of the terminal triazole moieties by using the azides **59**, **61**, **63**, **65**, **67**, **68** and respective alkyne intermediates *via* a CuAAC reaction, followed by side chain *N*-deprotection of the *N*-Pbf and *N*-Boc groups with CH_2Cl_2/H_2O/TFA.

Synthesis of the derivatives 35 – 36 from the azide 59:

The derivatives **35** and **36** (Scheme 2.23) were realized in yields of 55% and 57%, respectively over the two steps from the azide **59** by reaction with respective alkynes, $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O at rt for 16 h. The *N*-protected triazole derivatives so obtained were then subjected to *N*-Boc/*N*-Pbf deprotection with TFA and 20 equivalents of the H_2O in CH_2Cl_2 at rt for 16 h followed by the treatment with ethereal HCl and purification by precipitation (Scheme 2.23).



Compound	R	Yield (%)[a]
35		55
36		57

[a] Yields are reported over two steps from 59

Scheme 2.23 – Derivatization of the azide **59** *via* installation of the terminal triazole moiety followed by deprotection of the *N*-Pbf and *N*-Boc side chains protecting groups to give derivatives **35** and **36** as hydrochloride salts

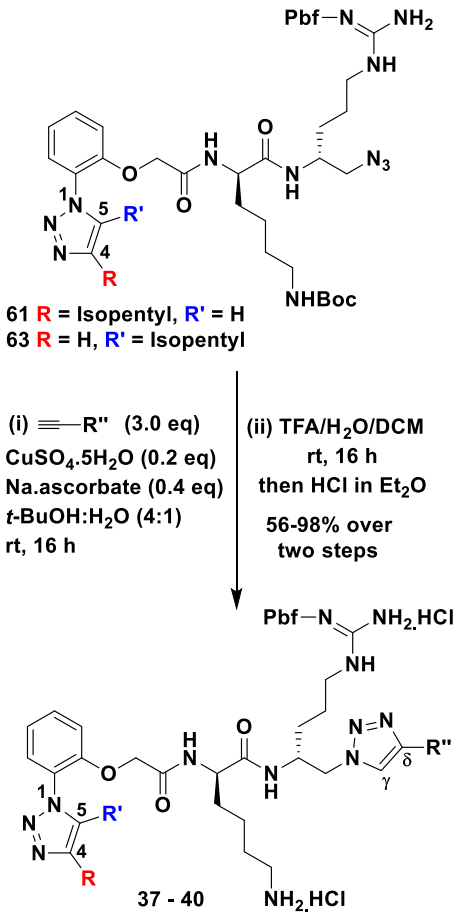
The ^{1}H NMR spectrum of the derivative **35** showed a resonance for the methylene of the benzyl unit as a singlet resonance at δ_H 4.03 (2H) and a multiplet resonance for the phenyl group at δ_H 7.50 – 7.14 (5H). The gHSQC spectrum of **35** showed that the two triazole ring protons at δ_H 8.74 (H5) and δ_H 8.23 (Hγ) correlated to the carbon resonances at δ_C 130.0 (C-5) and δ_C 128.1 (C-γ), respectively. The carbon resonances at δ_C 143.1 (C-4) and δ_C 130.3 (C-δ) were assigned to the quaternary carbons of the triazoles based on the gHMBC correlations of these resonances to those of their respective triazole ring protons [δ_H 8.74 (H5) and δ_H 8.23 (Hγ)]. The arginine peptide chain was confirmed from the gHMBC correlations of H-1' proton (δ_H 4.78) with carbon resonances C-2' (δ_C 50.9), C-3' (δ_C 29.7) and C-γ (δ_C 126.4), respectively. The H-2' proton (δ_H 4.28-4.19) correlated

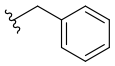
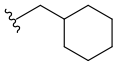
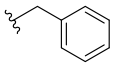
with carbon resonances C-1' (δ_C 57.2), C-3' (δ_C 29.7) and C-4' (δ_C 26.4). Furthermore, the H-5' proton (δ_H 2.92-2.84) correlated with carbon resonances C-3' (δ_C 29.7), C-4' (δ_C 26.4) and guanidine C=N (δ_C 158.7).

The protons and carbons of the lysine peptide chain were assigned based on analysis of the gHMBC spectrum which showed correlations of the Lys5 proton (δ_H 4.46-4.32) to carbon resonances Lys4 (δ_C 32.4), Lys3 (δ_C 24.3) and βC=O (δ_C 174.5). Furthermore, the Lys3 proton (δ_H 0.94-0.86) correlated to carbon resonances Lys1 (δ_C 40.7), Lys2 (δ_C 28.1) and Lys4 (δ_C 32.4). The molecular structure of the derivative **35** was further confirmed by the appearance of the ion peak at *m*/*z* 721.4046 in HRMS (ESI) that was assigned to the protonated molecular ion, $[M - 2HCl + H]^+$ (calculated for $C_{38}H_{49}N_{12}O_3$ 721.4045). The other derivative **36** in Scheme 2.23, was also fully characterized by NMR and HRMS analysis.

Synthesis of the derivatives 37 – 40 from the azides 61 and 63:

The derivatives **37 – 40** (Scheme 2.24) were realized in yields ranging from 56 – 98% over the two steps from the azides **61** and **63** by reaction with respective alkynes, $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O at rt for 16 h. The *N*-protected triazole derivatives so obtained were then subjected to *N*-Boc/*N*-Pbf deprotection with TFA and 20 equivalents of the H_2O in CH_2Cl_2 at rt for 16 h followed by the treatment with ethereal HCl and purification of the HCl salts by precipitation.



Compound	R	R'	R"	Yield (%)[a]
37	Isopentyl	H		56
38	H	Isopentyl		98
39	H	Isopentyl		64
40	H	Isopentyl		52

[a] Yields are reported over two steps from 61 and 63

Scheme 2.24 – Derivatization of the azides **61** and **63** *via* installation of the terminal triazole moiety followed by deprotection of the *N*-Pbf and *N*-Boc side chains protecting groups to give derivatives **37 – 40** as hydrochloride salts

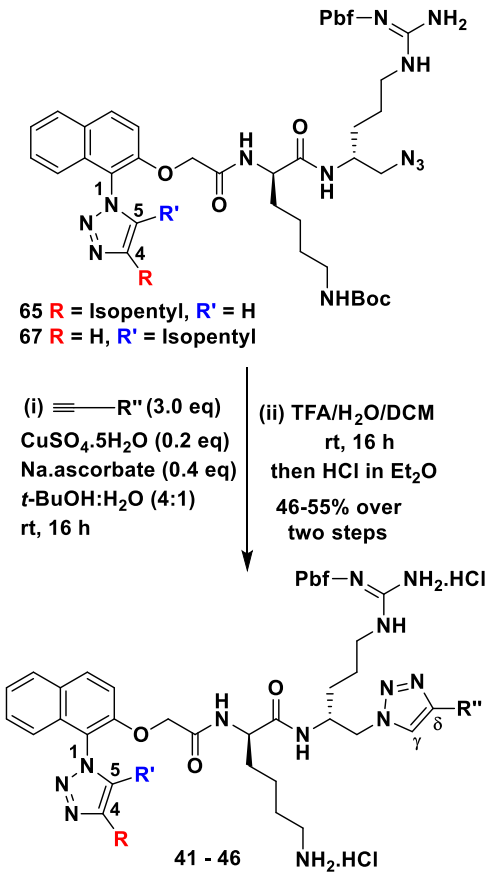
The ^{1}H NMR spectrum of the derivative **37** displayed characteristic resonances that were assigned to the methylene protons of the benzyl unit i.e., a singlet resonance at δ_H 4.12 (2H) and the multiplet resonances of the phenyl at δ_H 7.32 – 7.20 (5H). The gHSQC spectrum of **37** showed that the two singlet resonances assigned to the two triazole ring protons at δ_H 8.54 (H5) and δ_H 8.12 (Hγ) were correlated to the carbon resonances at δ_C 126.2 (C-5) and δ_C 125.9 (C-γ), respectively. The gHMBC spectrum allowed the carbon resonances at δ_C 137.5 (C-δ) and δ_C 133.5 (C-4) to be assigned to the quaternary carbons of the triazoles. The molecular structure of the derivative **37** was verified by the appearance of the ion peak at *m/z* 773.1240 in HRMS (ESI) that was assigned to the

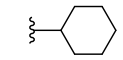
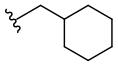
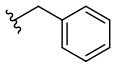
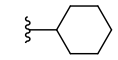
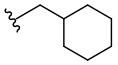
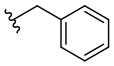
protonated molecular ion, $[M + H]^+$ (calcd for $C_{36}H_{55}N_{12}O_3Cl_2$ 773.1236). The other derivatives **38** – **40** in Scheme 2.24, were also fully characterized by NMR and HRMS analysis.

Synthesis of the derivatives 41 – 46 from the azides 65 and 67:

The derivatives **41 – 46** (Scheme 2.25) were realized in yields ranging from 46 – 55% over the two steps from the azides **65** and **67** by reaction with respective alkynes, $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O at rt for 16 h. The *N*-protected triazole derivatives so obtained were then subjected to *N*-Boc/*N*-Pbf deprotection with TFA and 20 equivalents of the H_2O in CH_2Cl_2 at rt for 16 h followed by the treatment with ethereal HCl and purification by precipitation.

The ^{1}H NMR spectrum of the derivative **41** showed characteristic resonances (11H) that were assigned to the cyclohexyl group at δ_H 2.84 – 2.78 (m, 1H), δ_H 1.74 – 1.60 (m, 5H) and δ_H 1.48 – 1.21 (m, 5H). The gHSQC spectrum showed that the two singlet resonances which were assigned to the two triazole ring protons at δ_H 8.30 (H5) and δ_H 8.29 (Hγ) correlated to the carbon resonances at δ_C 125.5 (C-5) and δ_C 125.1 (C-γ), respectively. The carbon resonances at δ_C 148.7 (C-4) and δ_C 147.6 (C-δ) were assigned to the quaternary carbons of the triazoles based on their gHMBC correlations.



Compound	R	R'	R''	Yield (%)[a]
41	Isopentyl	H		46
42	Isopentyl	H		52
43	Isopentyl	H		52
44	H	Isopentyl		55
45	H	Isopentyl		54
46	H	Isopentyl		53

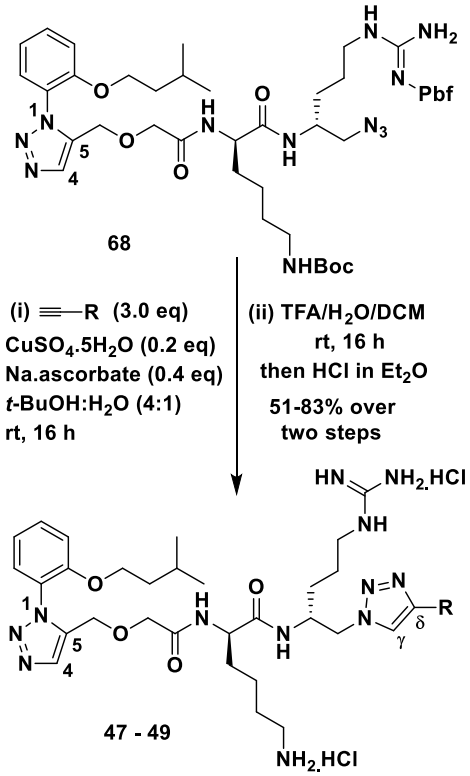
[a] Yields are reported over two steps from 65 and 67

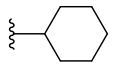
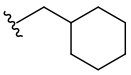
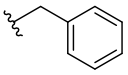
Scheme 2.25 – Derivatization of the azides **65** and **67** *via* installation of the terminal triazole moiety followed by deprotection of the *N*-Pbf and *N*-Boc side chains protecting groups to give derivatives **41 – 46** as hydrochloride salts

The molecular structure of the derivative **41** was further established by the appearance of the ion peak at *m/z* 743.4866 in HRMS (ESI) that was assigned to the protonated molecular ion, $[M - 2HCl + H]^+$ (calcd for $C_{39}H_{59}N_{12}O_3$ 743.4833). The other derivatives **42** – **46** in Scheme 2.25, were also fully characterized by NMR and HRMS analysis. The derivatives **44 – 46** also displayed amide rotamers (i.e. *anti* and *syn*) which was evident by the presence of smaller, additional resonances in their ^1H and ^{13}C NMR spectra.

Synthesis of the derivatives 47 – 49 from the azide 68:

The derivatives **47 – 49** (Scheme 2.26) were realized in yields ranging from 51 – 83% over the two steps from the azide **68** by reaction with respective alkynes, $CuSO_4 \cdot 5H_2O$ and sodium ascorbate in *t*-BuOH/H_2O at rt for 16 h. The *N*-protected triazole derivatives so obtained were then subjected to *N*-Boc/*N*-Pbf deprotection with TFA and 20 equivalents of the H_2O in CH_2Cl_2 at rt for 16 h followed by the treatment with ethereal HCl and purification of HCl salts by precipitation.



Compound	R	Yield (%)[a]
47		61
48		83
49		51

[a]Yields are reported over two steps from 68

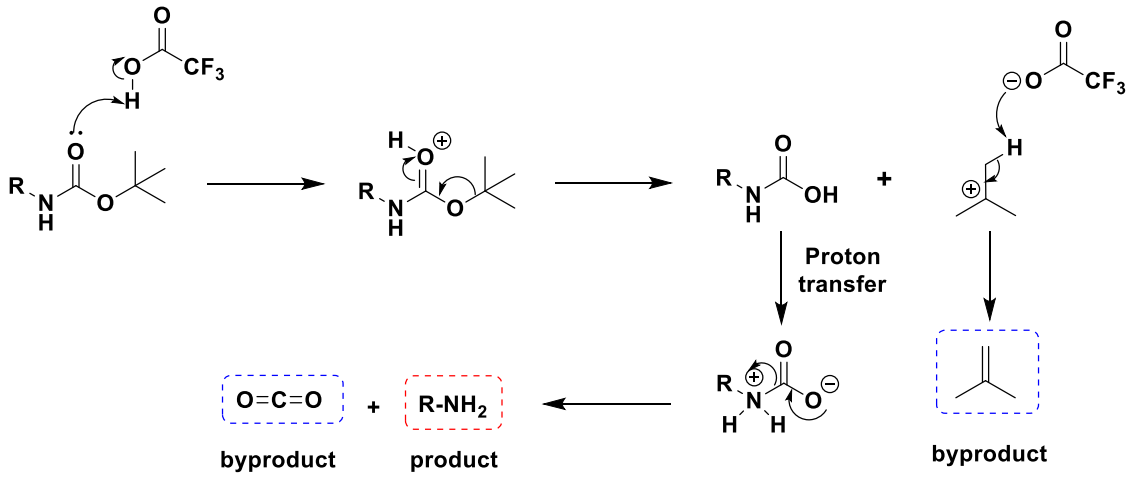
Scheme 2.26 – Derivatization of the azide **68** *via* installation of the terminal triazole moiety followed by deprotection of the *N*-Pbf and *N*-Boc side chains protecting groups to give derivatives **47** – **49** as hydrochloride salts

The ^1H NMR spectrum of **47** showed characteristic resonances (11H) for the cyclohexyl group at δ_H 2.74 (s, 1H), δ_H 1.78 – 1.55 (m, 5H) and δ_H 1.54 – 1.37 (m, 5H).

The ^{1}H NMR spectrum of **47** showed two overlapping singlet resonances at δ_H 8.05 (H4 and Hγ) which were assigned to the two triazole ring protons from their correlations to the carbon resonances at δ_C 128.4 and δ_C 120.61. The carbon resonances at δ_C 132.06 (C-5) and 132.02 (C-δ) were assigned to the two quaternary carbons of the triazoles, however they could not be individually assigned as they could not be distinguished in the HMBC spectrum as both triazole protons had the same chemical shift (δ_H 8.05). The molecular structure of the derivative **47** was confirmed by the appearance of the ion peak at *m*/*z* 723.4810 in HRMS (ESI) that was assigned to the protonated molecular ion, $[M - 2HCl + H]^+$ (calcd for $C_{36}H_{59}N_{12}O_4$ 723.4782). The other derivatives **48** and **49** in Scheme 2.26, were also fully characterized by NMR and HRMS analysis.

Mechanism of *N*-Boc deprotection:

The *N*-Boc group is particularly sensitive to acids and often uses TFA as the deprotection agent producing the required free amine and by-products i.e. 2-methyl-1-propene and CO_2 (Scheme 2.27).[61] The acidic deprotection mechanism starts with protonation of the carbonyl oxygen of carbamate followed by heterolytic cleavage of the bond between O–C$(CH_3)_3$ to produce the carbamic acid and the stable *t*-butyl carbocation (Scheme 2.27) that is converted into 2-methyl-1-propene after proton abstraction by trifluoroacetate anion. Decomposition of the carbamic acid proceeds spontaneously *via* protonation of carbamic acid nitrogen to give the free amine product which rapidly rotonates with excess TFA and CO_2 as volatile by product.[61] The overall reaction time is 10 – 15 minutes.



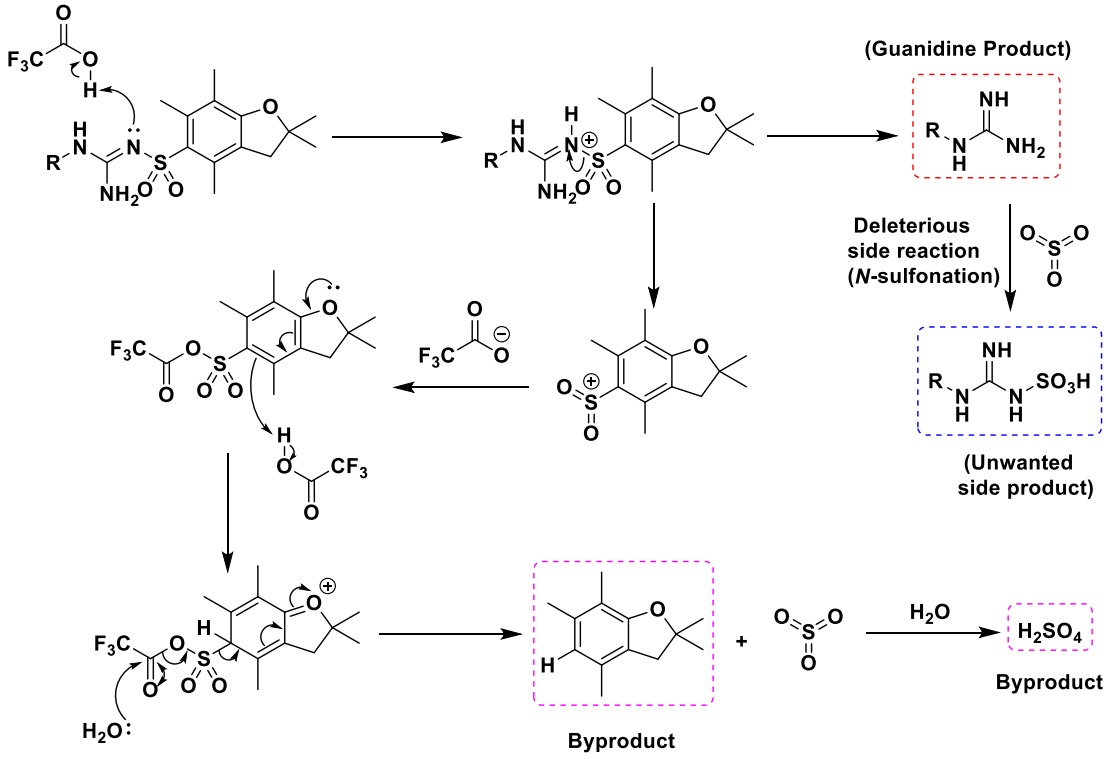
Scheme 2.27 – *N*-Boc deprotection with TFA to produce the required product amine (red) and by-products (blue)

Mechanism of *N*-Pbf deprotection:

The deprotection of *N*-Pbf group in the presence of acid requires a longer reaction time compared to *N*-Boc deprotection and an electrophile SO_3 scavenger, in this case H_2O is needed to control the formation of unwanted products *via* reaction with SO_3.[68]

The deprotection of *N*-Pbf (Scheme 2.28) starts with protonation of the guanidino nitrogen followed by heterolytic cleavage of the N – S bond to produce the required guanidine as the TFA salt and byproduct arylsulfonyl cation.[66, 68-69] The arylsulfonyl cation reacts with the trifluoroacetate anion to form an intermediate, followed by protonation, with hydrolysis initiating decomposition to produce 2,2,4,6,7-pentamethyldihydrobenzo[*b*]furan and SO_3.[68-69] The electrophile SO_3 can reacts with the deprotected guanidine residue and form a sulfamic acid compound,[67] therefore water as a scavenger is required to stop the formation of sulfamic acid. The overall reaction times were 12 – 16 h for complete deprotection of the N-Pbf protecting group.

Acidic conditions were needed for the deprotection of both the *N*-Boc and *N*-Pbf protecting groups to produce the crude TFA-amine salt after evaporation of the reaction mixture solvent, followed by anion exchange with anhydrous HCl in diethyl ether to obtain final derivatives as HCl salt for antibacterial screening. The final HCl salts needed to be triturated in diethyl ether solvent repeatedly until the benzofuran by-product was removed.



Scheme 2.28 – Deprotection of the *N*-Pbf group to produce product guanidine (red) and by-products (pink).

In conclusion, a total of 29 novel antibacterial compounds were synthesized and fully characterized. The synthesis of these derivatives involved the synthesis of 96 intermediate compounds (i.e. 87 novel intermediate compounds and 9 known compounds) using key reactions such as the one pot process of azidation and Cu-catalyzed

cycloaddition reaction, Ru-catalyzed cycloaddition reaction, Mg-promoted cycloaddition reaction, Appel reaction, peptide coupling and deprotection with TFA.

3.0 – Biological and pharmacological assays: results and discussion

3.1 – Background information

This chapter reports the results of the *in vitro*, *in vivo* and pharmacological assays performed on the deprotected phenyltriazole and naphthalenetriazole peptide derivatives, as their HCl-salts, which were described in Chapter 2. These compounds were screened in three stages (Figure 3.1).

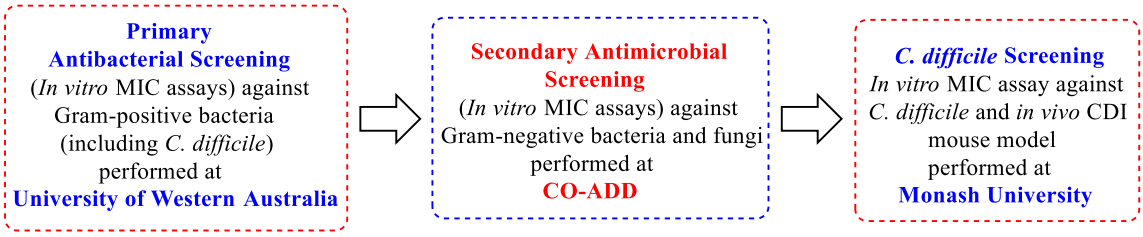


Figure 3.1 – Summary of the biological screening process used to ascertain antimicrobial activity

Primary antibacterial testing:

Primary antibacterial testing (*in vitro* MIC assays) against Gram-positive bacteria including *C. difficile* (Figure 3.1) was performed with project collaborators at the University of Western Australia.[†]

Secondary antibacterial testing:

Secondary antibacterial testing (*in vitro* MIC assays) against Gram-negative bacteria including fungi (Figure 3.1) and a cytotoxicity assay was performed by CO-ADD.[#]

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† Prof. Thomas V. Riley, Dr. Katherine A. Hammer and Dr. Dan Knight.

Community for Open Antimicrobial Drug Discovery (www.co-add.org) – funded by the Wellcome Trust (UK) and The University of Queensland.

***C. difficile* screening:**

Compounds that displayed significant MIC values in the primary and secondary testing stages were moved to third round *in vitro* MIC testing against the hypervirulent *C. difficile* strain (RT027 – M7404) with project collaborators at Monash University.[§] The compounds that performed best *in vitro* were then selected for testing in an *in vivo* murine model of CDI to determine the efficacy and viability of the compound as a potential CDI inhibitor (Figure 3.1).

3.2 – Bacterial species used for *In vitro* assays (MIC)

The MIC assays were performed against four Gram-positive, or four Gram-negative bacterial species and two fungal species.

Gram +ve Bacterial Species	**Primary Screening (UWA)**	**Secondary Screening (CO-ADD)**	***C. difficile* screening (Monash)**
Staphylococcus aureus	ATCC 29213	-	-
	NCTC 10442 (MRSA)	ATCC 43300 (MRSA)	-
Clostridium difficile	ATCC 700057	-	M7404 (RT027)
			NSW132 (RT027)
Enterococcus faecalis	ATCC 29219	-	-
Streptococcus pneumoniae	ATCC 49619	-	-
Gram -ve Bacterial Species			
Escherichia coli	ATCC 25922	ATCC 25922	-
Klebsiella pneumoniae	-	ATCC 700603 (MDR)	-
Acinetobacter baumannii	-	ATCC 19606	-
Pseudomonas aeruginosa	-	ATCC 27853	-
Fungal Species			
Candida albicans	-	ATCC 90028	-
Cryptococcus neoformans	-	ATCC 208821	-

Table 3.1 – Bacterial and fungal species that were tested against in the various *in vitro* MIC assays

§ Prof. Dena Lyras, Dr. Melanie Hutton, Dr. Amy King and Dr. Yogi Srikhanta

The compounds were screened against these bacterial pathogens and fungi to measure their broad-spectrum antimicrobial activity i.e. activity against five of the six ESKAPE pathogens[5] (i.e. ***E****nterococcus faecium*, ***S****taphylococcus aureus*, ***K****lebsiella pneumoniae*, ***A****cinetobacter baumannii*, ***P****seudomonas aeruginosa* and ***E****nterobacter* spp.) and their activity against *C. difficile* strains to find potential CDI inhibitors.

3.2.1 – General methodology for *in vitro* assays and cytotoxicity assays

***In vitro* assays**: For primary antibacterial screening, multi-well micro-titer plates were loaded with immunized and developed medium and different concentrations of the compounds to be tested. Testing concentrations of the compounds ranged from 0.125 μg/mL to 128 μg/mL in DMSO were typically utilized. The plates were cultured, and the resultant bacterial growth inhibition was determined.

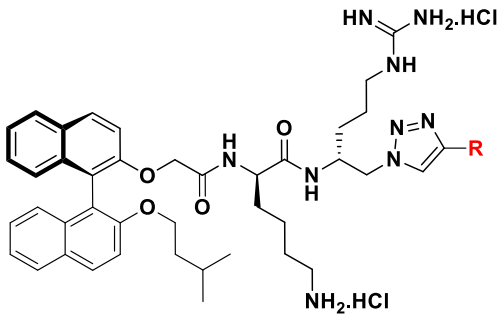
For testing at CO-ADD, initially only one concentration i.e. 32 μg/mL of every compound was analyzed to verify antimicrobial potency. Compounds that exhibited potency at this concentration then progressed to a comprehensive MIC test. Colistin and vancomycin were utilized as positive controls for both Gram-positive and Gram-negative bacteria. Fluconazole was used as the positive control for fungi.

Cytotoxicity assays: The cytotoxicity of every compound was evaluated at CO-ADD against human embryonic kidney cells (HEK-293 – ATCC CRL – 1573) by using tamoxifen as a control. The cytotoxicity data is described as the concentration needed to

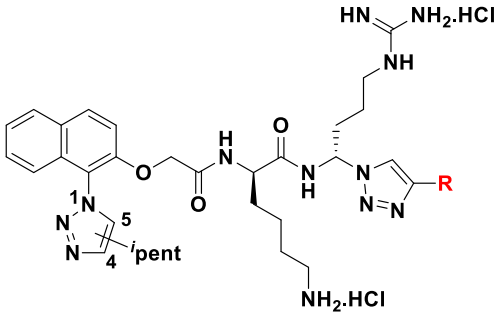
inhibit kidney cell growth by 50% (CC_{50}). The experimental procedures for the various MIC tests and cytotoxicity test are discussed in Section 6.4.1.

3.2.2 – SAR trends (MIC assay results from UWA and Monash)

Twenty-nine compounds were prepared from 11 distinctive scaffolds. In the SAR analysis, the antibacterial activity of the twenty-nine derivatives were compared against the antibacterial activity of AVX 13616 (lead compound **1**) and the previously synthesized compounds **18 – 20** using vancomycin as the control.



18 - 20



41 - 46

Compound	R	^{i}pentyl position	*S. aureus* ATCC 29213	*S. aureus* NCTC 10442	*E. faecalis* ATCC 29212	*S. pneumoniae* ATCC 49619	*E. coli* ATCC 25922	*C. difficile* ATCC 700057	*C. difficile* 132 (RT027)
1	-	-	**2**	**2**	**2**	**2**	**16**	**8**	**16**
18	Cy	-	2	4	2	4	8	16	16
19	CH_2Cy	-	4	4	4	8	8	32	16
20	Bn	-	2	4	4	2	8	16	16
41	Cy	C-4	16	16	32	16	64	**8**	**8**
42	CH_2Cy	C-4	8	8	8	16	128	16	16
43	Bn	C-4	8	8	8	8	32	**8**	**8**
44	Cy	C-5	16	16	64	**4**	64	128	128
45	CH_2Cy	C-5	8	**4**	16	**4**	64	32	32
46	Bn	C-5	16	16	64	**4**	128	128	128
Vancomycin	-	-	1	1	4	1	>16	0.5	0.5

Table 3.2 – Primary screening data for compounds **41 – 46** in μg/mL (MIC values). **RT 700057** tested at UWA and **RT027** tested at Monash (The data for compounds **18** – **20** from references 47 and 53)

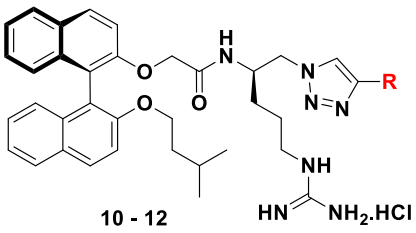
Compounds **41 – 46** were designed from compounds **18 – 20** by modifying the binaphthyl moiety to a naphthyltriazole base structure with variations of the isopentyl

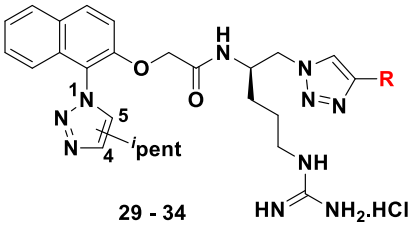
substituent at the C-4 and C-5 positions of the triazole ring of the naphthyl-triazole moiety (Table 3.2). Compound **18** (R = Cy) displayed antibacterial activities (MIC values) of 2 μg/mL and 4 μg/mL against the *S. aureus* strains, whereas the modified compounds **41** (R = Cy) and **44** (R = Cy) displayed MIC values of 16 μg/mL against these strains. Compounds **18 – 20** and **45** (R = CH_2Cy) showed similar antibacterial activities (MIC) of 4 μg/mL against *S. aureus* (NCTC 10442). Compound **19** (R = CH_2Cy) exhibited a MIC value of 4 μg/mL against the *S. aureus* strains, whereas the modified compound **42** (R = CH_2Cy) displayed a MIC of 8 μg/mL against these strains. Apart from compound **45**, all other compounds (**41 – 44** and **46**) with an isopentyl substituent at the C-4 or C-5 position showed little difference in activity against the *S. aureus* strains. In general, the modified compounds were less active than compounds **18 – 20** (Table 3.2) against the *S. aureus* strains.

Compounds **42** (R = CH_2Cy) and **43** (R = Bn) exhibited MIC values of 8 μg/mL against *E. faecalis*, whereas the other modified compounds **41** (R = Cy) and **44 – 46** (Table 3.2) had MIC values of 32 μg/mL and 64 μg/mL, respectively against *E. faecalis*.

Compound **20** (R = Bn) displayed the best antibacterial activity (MIC = 2 μg/mL) against *S. pneumoniae* among the compounds **18 – 20** (Table 3.2), while the modified compounds **44 – 46**, having a C-5 substituent all showed similar and significant antibacterial activity against this bacterium (MIC = 4 μg/mL).

Compounds **18** (R = Cy) and **20** (R = Bn) displayed MIC values of 16 μg/mL against the two *C. difficile* strains, however the modified compounds **41** (R = Cy) and **43** (R = Bn) showed slightly better MIC values of 8 μg/mL against these strains (Table 3.2).





Compound	R	^{i}pentyl position	*S. aureus* ATCC 29213	*S. aureus* NCTC 10442	*E. faecalis* ATCC 29212	*S. pneumoniae* ATCC 49619	*E. coli* ATCC 25922	*C. difficile* ATCC 700057	*C. difficile* 132 (RT027)
1	-	-	**2**	**2**	**2**	**2**	**16**	**8**	**16**
10	Cy	-	2	2	4	8	>128	16	32
11	CH_2Cy	-	4	4	4	8	>128	32	32
12	Bn	-	2	2	4	16	128	32	32
29	Cy	C-4	8	8	16	16	128	128	>128
30	CH_2Cy	C-4	**4**	**4**	8	8	32	32	32
31	Bn	C-4	**4**	**4**	**4**	**4**	64	32	32
32	Cy	C-5	8	8	8	8	32	32	32
33	CH_2Cy	C-5	**4**	**4**	8	8	16	32	64
34	Bn	C-5	8	8	16	**4**	64	64	>128
Vancomycin	-	-	1	1	4	1	>16	0.5	0.5

Table 3.3 – Primary screening data for compounds **29 – 34** in µg/mL (MIC values). **RT 700057** tested at UWA and **RT027** tested at Monash (The data for compounds **10** – **12** from references 47 and 53)

Compounds **29 – 34** were designed from the monocationic compounds **10 – 12** by modifying the binaphthyl moiety to a naphthyltriazole base structure with variations of the isopentyl substituent at the C-4 and C-5 position of the triazole ring of the naphthyltriazole moiety (Table 3.3). Compound **10** (R = Cy) displayed antibacterial

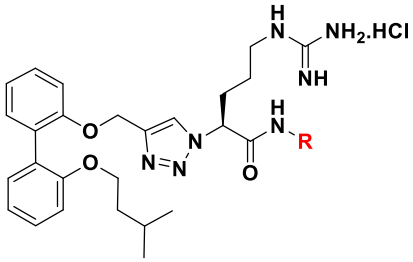
activity (MIC) of 2 μg/mL against *S. aureus* strains, whereas the modified compounds **29** (R = Cy) and **32** (R = Cy) had MIC values of 8 μg/mL against the *S. aureus* strains. Compounds **11** (R = CH_2Cy), **30** (R = CH_2Cy) and **33** (R = CH_2Cy) showed similar antibacterial activities against the *S. aureus* strains (MIC = 4 μg/mL). Compound **12** (R = Bn) exhibited antibacterial activity (MIC) of 2 μg/mL against both *S. aureus* strains, whereas the modified compounds **31** (R = Bn) and **34** (R = Bn) had MIC values of 4 μg/mL and 8 μg/mL, respectively against these strains.

Compounds **10 – 12** exhibited MIC values of 4 μg/mL against *E. faecalis*, whereas the modified compound **31** (R = Bn) displayed similar antibacterial activity with the others being less active.

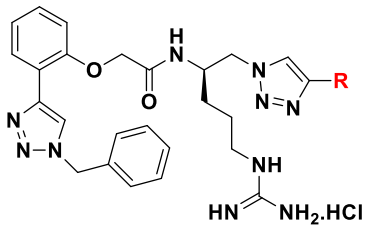
Compounds **10 – 11** and **30**, **32 – 33** showed similar antibacterial activities against *S. pneumoniae* (MIC = 8 μg/mL). While compounds **31** (R = Bn) and **34** (R = Bn) displayed better antibacterial activities (MIC = 4 μg/mL) against *S. pneumoniae* and compounds **12** (R = Bn) and **29** (R = Cy) showed the poorest activities (MIC = 16 μg/mL).

None of the compounds in Table 3.3 were particularly active against *E. coli* (MIC = 16 – >128 μg/mL), while compound **33** (R = CH_2Cy) showing the best activity (MIC = 16 μg/mL). None of the compounds in Table 3.3 showed significant activities against the two *C. difficile* strains (MIC = 32 – >128 μg/mL).

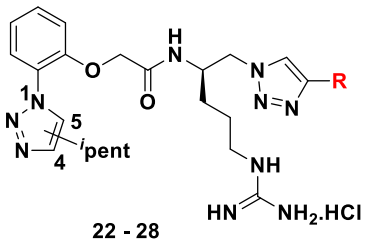
A comparison of Tables 3.2 and 3.3 indicated that, in general the monocationic compounds in Table 3.3 had better activities against *S. aureus* than the dicationic compounds in Table 3.2. The C-4 substituted dicationic compounds **41** and **43** were the most active against the *C. difficile* strains of the compounds from these two Tables.



6 - 8



21



22 - 28

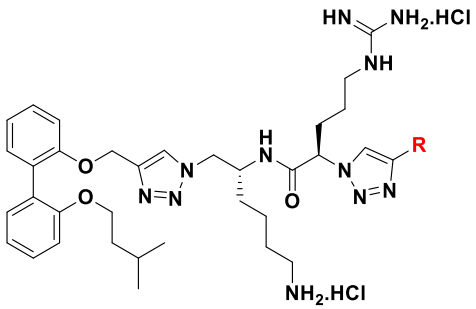
Compound	R	^{i}pentyl position	*S. aureus* ATCC 29213	*S. aureus* NCTC 10442	*E. faecalis* ATCC 29212	*S. pneumoniae* ATCC 49619	*E. coli* ATCC 25922	*C. difficile* ATCC 700057	*C. difficile* 132 (RT027)
1	-	-	**2**	**2**	**2**	**2**	**16**	**8**	**16**
6	Cy	-	4	4	4	4	32	8	8
7	CH_2Cy	-	4	4	4	4	32	8	8
8	Bn	-	4	8	8	4	32	32	16
21	CH_2Cy	-	64	64	64	32	>128	64	64
22	Cy	C-4	32	32	32	16	128	32	64
23	Cp	C-4	32	32	32	16	128	32	64
24	4-F-Ph	C-4	32	32	32	16	128	32	64
25	Bn	C-4	32	32	32	16	128	32	64
26	Cy	C-5	32	32	32	32	>128	128	128
27	CH_2Cy	C-5	16	16	16	16	128	64	64
28	Bn	C-5	32	32	32	32	>128	32	32
Vancomycin	-	-	1	1	4	1	>16	0.5	0.5

Table 3.4 – Primary screening data for compounds **21 – 28** in μg/mL (MIC values). **RT 700057** tested at UWA and **RT027** tested at Monash (The data for compounds **6** – **8** from reference 89)

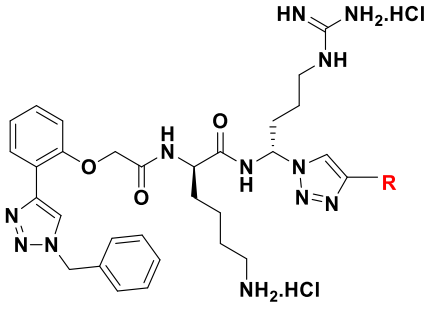
The monocationic compounds **21 – 28** were designed from compounds **6 – 8** by modifying the biphenyl to a phenyl-triazole base structure with one derivative having a *N*-benzyl substituent rather than an isopentyl substituent on the triazole ring (Table 3.4). This modification led to a decrease in the antibacterial activities from 4 – 32 μg/mL to 16 – >128 μg/mL against both Gram-positive and Gram-negative bacteria.

Primary screening data for 35-40 and 47-49

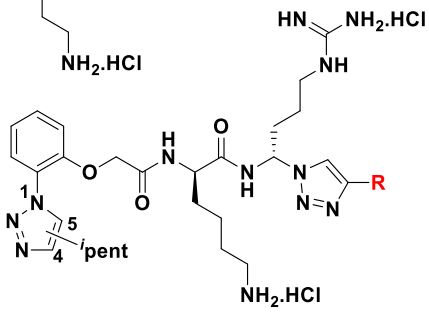
The dicationic compounds **35 – 40** were designed from compounds **14 – 16** by modifying the biphenyl group to a phenyl-triazole base structure with **35** and **36** having a *N*-benzyl substituent on the triazole ring, while compounds **37 – 40** had an isopentyl substituent at the C-4 and C-5 positions of the triazole ring of the phenyl-triazole moiety. These modifications led to a decrease in the antibacterial activity from 2 – 32 μg/mL to 8 – >128 μg/mL against both Gram-positive and Gram-negative bacteria (Table 3.5).



14 - 16



35 - 36

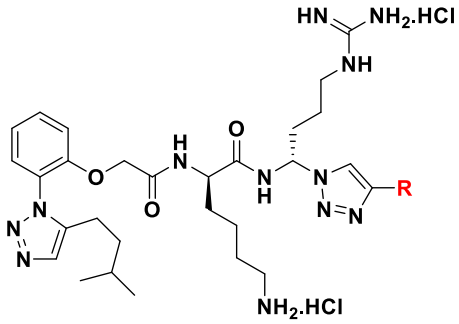


37 - 40

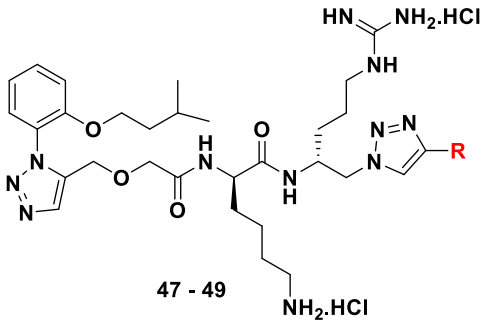
Compound	R	^{i}pentyl position	*S. aureus* ATCC 29213	*S. aureus* NCTC 10442	*E. faecalis* ATCC 29212	*S. pneumoniae* ATCC 49619	*E. coli* ATCC 25922	*C. difficile* ATCC 700057	*C. difficile* 132 (RT027)
1	-	-	**2**	**2**	**2**	**2**	**16**	**8**	**16**
14	Cy	-	8	8	8	2	16	8	16
15	CH_2Cy	-	4	4	4	2	16	8	16
16	Bn	-	8	8	8	4	32	32	16
35	Bn	-	32	32	64	16	128	128	128
36	CH_2Cy	-	32	32	64	16	128	128	128
37	Bn	C-4	32	32	64	16	128	128	128
38	Cy	C-5	16	32	16	8	64	64	64
39	CH_2Cy	C-5	128	128	>128	128	>128	128	>128
40	Bn	C-5	32	64	32	16	128	64	32
Vancomycin	-	-	1	1	4	1	>16	0.5	0.5

Table 3.5 – Primary screening data for compounds **35 – 4**0 in µg/mL (MIC values). **RT 700057** tested at UWA and **RT027** tested at Monash (The data for compounds **14** – **16** from reference 89).

The dicationic compounds **47 – 49** were designed from compounds **38 – 40** by switching the position of the phenyl and triazole rings. These modifications did not increase the antibacterial activity against the two Gram-positive *C. difficile* strains (Table 3.6). The testing of compounds **47 – 49** is in progress.



38 - 40



47 - 49

Compound	R	^{i}pentyl position	*S. aureus* ATCC 29213	*S. aureus* NCTC 10442	*E. faecalis* ATCC 29212	*S. pneumoniae* ATCC 49619	*E. coli* ATCC 25922	*C. difficile* ATCC 700057	*C. difficile* 132 (RT027)
1	-	-	**2**	**2**	**2**	**2**	**16**	**8**	**16**
38	Cy	C-5	16	32	16	8	64	64	64
39	CH_2Cy	C-5	128	128	>128	128	>128	128	>128
40	Bn	C-5	32	64	32	16	128	64	32
47	Cy	C-5	-	-	-	-	-	-	>40
48	CH_2Cy	C-5	-	-	-	-	-	-	>40
49	Bn	C-5	-	-	-	-	-	-	>40
Vancomycin	-	-	1	1	4	1	>16	0.5	0.5

Table 3.6 – Primary screening data for compounds **47 – 49** in μg/mL (MIC values). **RT 700057** tested at UWA and **RT027** tested at Monash.

Overall SAR trend:

The dicationic binaphthyl derivatives **18 – 20** showed MIC values in the range 2 – 8 μg/mL against all Gram-positive and Gram-negative bacteria tested, except for *C. difficile* (MIC = 16 – 32 μg/mL), while the modified derivatives **41 – 46** displayed MIC values in the range of 4 – 16 μg/mL against all Gram-positive bacteria, including *C. difficile* (Table 3.2). Similarly, the monocationic binaphthyl derivatives **10 – 12** exhibited MIC values in the range of 2 – 8 μg/mL against all Gram-positive bacteria (except *C. difficile*), whereas the modified derivatives **29 – 34** exhibited MIC values in the range of 4 – 8 μg/mL (Table 3.3). The dicationic derivatives **41** and **43** both exhibited MIC values of 8 μg/mL against the *C. difficile* but none of the monocationic derivatives were as active.

The above results suggest that most of the modified naphthyl dicationic derivatives **41 – 46** (Table 3.2) were less active (MIC = 8 – 64 μg/mL) against Gram positive bacteria (except *C. difficile*), whereas modified naphthyl monocationic derivatives **29 – 34** (Table 3.3) were more active (MIC = 4 – 8 μg/mL) against Gram positive bacteria (except *C. difficile*). Furthermore, the modified naphthyl dicationic derivatives **41** and **43** (Table 3.2) were more active (MIC = 8 μg/mL) against Gram positive *C. difficile* strains, whereas the modified naphthyl monocationic derivatives **29 – 34** (Table 3.3) were not active (MIC = 32 – >128 μg/mL) against these *C. difficile* strains. These results promoted compound **41** to be chosen for *in vivo* studies in a murine model against CDI.

Furthermore, the dicationic biphenyl derivatives **14 – 16** had MIC values in the range of 2 – 8 μg/mL against all Gram-positive bacteria, whereas the modified derivatives **35 – 40** and **47 – 49** exhibited MIC values in the range of 8 – 32 μg/mL (Tables 3.5 and

3.6). Similarly, the monocationic biphenyl derivatives **6 – 8** exhibited antibacterial activity (MIC) in the range of 4 **–** 8 μg/mL against all Gram-positive bacteria but the modified derivatives **21 – 28** exhibited MIC values in the range of 32 **–** 64 μg/mL (Table 3.4). These results suggested that changing a phenyl ring of the biphenyl moiety with a triazole ring often led to a decrease in the antibacterial activity.

The potency of naphthalene dicationic derivative **41** against the *C. difficile* (MIC = 8 μg/mL; Table 3.2) was particularly better than the potency of the related naphthalene monocationic derivative **29** (MIC = 32 μg/mL; Table 3.3).

The compounds **41** – **43** displayed antimicrobial activity against the Gram-positive bacteria in the range of 8 – 16 μg/mL but were not active against *E. coli* (Table – 3.2). Additionally, two compounds (**41** and **43**) were recognized as potential CDI inhibitors due to their *in vitro* activities against the *C. difficile* strains (MIC = 8 μg/mL) and their good water solubility profiles (see section 3.2.6 and Table – 3.9).

Out of the 29 compounds tested, only 12 derivatives from the mono- and di-cationic derivatives of 1,4-disubstituted-naphthyl-1,2,3-triazoles **29 – 31** and **41 – 43** and 1,5-disubstituted-naphthyl-1,2,3-triazoles **32 – 34** and **44 – 46** exhibited MIC values in range of 4 **–** 16 μg/mL against *S. aureus* in secondary screening. None of the compounds exhibited antibacterial activity against Gram-negative bacteria but surprisingly a few compounds, **29 – 30**, **33** and **43 – 45**, exhibited weak antifungal activity (MIC = 8 **–** 32 μg/mL) against *C. albicans* and *C. neoformans* (Tables 3.7). The compounds **41** and **42** showing MIC values of 4 μg/mL against *C. neoformans* (Tables 3.7).

Secondary Screening Data

Compound	*S. aur.*	*P. aer.*	*K. pneu.*	*A. bau.*	*E. coli*	*C. alb.*	*C. neo.*	CC_{50}
21	16	>32	>32	>32	>32	>32	32	>32
22	32	>32	>32	>32	>32	>32	>32	>32
23	32	>32	>32	>32	>32	>32	>32	>32
24	32	>32	>32	>32	>32	>32	>32	>32
25	32	>32	>32	>32	>32	>32	>32	>32
26	32	>32	>32	>32	>32	>32	>32	>32
27	16	>32	>32	>32	>32	>32	>32	>32
28	32	>32	>32	>32	>32	>32	>32	>32
29	8	>32	>32	>32	32	32	16	32
30	**4**	>32	>32	>32	32	16	8	32
31	8	>32	>32	>32	32	32	16	32
32	8	32	>32	32	32	32	>32	21.9
33	**4**	>32	>32	32	32	16	8	>32
34	16	>32	>32	>32	>32	32	>32	23.5
35	16	>32	>32	>32	>32	32	>32	>32
36	32	>32	>32	>32	>32	32	>32	>32
37	16	>32	>32	>32	32	16	32	>32
38	16	>32	>32	>32	>32	16	16	>32
39	32	>32	>32	>32	>32	>32	16	32
40	32	>32	>32	>32	>32	16	16	>32
41	8	32	>32	32	32	>32	**4**	32
42	**4**	16	32	32	32	32	**4**	16
43	8	32	>32	32	32	>32	8	32

Table 3.7 – Secondary antimicrobial screening data in μg/mL (MIC and CC_{50} values).

Secondary Screening Data

Compound	*S. aur.*	*P. aer.*	*K. pneu.*	*A. bau.*	*E. coli*__	*C. alb.*__	*C. neo.*	CC_{50}
44	8	32	>32	32	32	>32	8	>32
45	8	32	>32	32	32	32	8	>32
46	16	32	>32	>32	32	>32	>32	>32

Table 3.7 (Continued) – Secondary antimicrobial screening data in μg/mL (MIC and CC_{50} values).

Most of the compounds were tested for partial cytotoxicity by using the same concentration (32 μg/mL) that was utilized for the initial MIC testing at CO-ADD. Compounds are usually analyzed for cytotoxicity at significantly higher concentrations. This low-concentration analysis allows the calculation of CC_{50} data (i.e. the concentration of drug which is cytotoxic to 50% of cells) via curve-fitting and extrapolation of the low-concentration cytotoxicity data. Some of the potent derivatives were unable to display significant cytotoxicity at low concentrations and hence curve fitting was impossible; a CC_{50} value could not be inferred from the data. These compounds have recorded a CC_{50} value > 32 μg/mL as they are unable to display significant cytotoxicity at the verified concentrations. Cytotoxicity tests were achieved in replicate and the lower CC_{50} data is reported in Table 3.7.

Most of the compounds showed little cytotoxicity with **32**, **34** and **42** exhibiting cytotoxicity (CC_{50}) at 21.9 μg/mL, 23.5 μg/mL and 16 μg/mL, respectively (Table 3.7). The di-cationic compounds **35 – 41** and **43** – **46** displayed little cytotoxicity (CC_{50} >32

μg/mL); but compound **42** showed significant cytotoxicity (CC_{50} – 16 μg/mL). Notably, the lead compound **41** (MIC **–** 8 μg/mL against *C. difficile*) displayed moderate cytotoxicity (CC_{50} = 32 μg/mL).

(CO-ADD) Secondary Screening - Control Data:

In the CO-ADD screening, vancomycin and colistin were used as positive controls for the Gram-positive and Gram-negative bacteria, respectively (Table 3.8). Fluconazole was used as a positive control for *C. albicans* and *C. neoformans* (Table 3.8). Tamoxifen was used as a positive control for cytotoxicity assay (Table 3.8). The antibiotics were provided in 4 concentrations, with 2 above and 2 below its MIC value, and plated into the first 8 wells of column 23 of the 384 **–** well NBS plates.

Secondary Screening - Control Data

Compound	*S. aureus* ATCC 43000 (MRSA)	*P. aeruginosa* ATCC 27853	*K. pneumoniae* ATCC 700603	*A. baumannii* ATCC 19606	*E. coli* ATCC 25922	*C. albicans* ATCC 90028	*C. neoformans* ATCC 208821	Cytotoxicity (CC_{50}) (HEK-293) ATCC CRL-1573
Vancomycin	1	>32	>32	>32	>32	-	-	-
Colistin	>32	0.25	0.25	0.25	0.125	-	-	-
Fluconazole	-	-	-	-	-	0.125	0.25	-
Tamoxifen	-	-	-	-	-	-	-	13.06

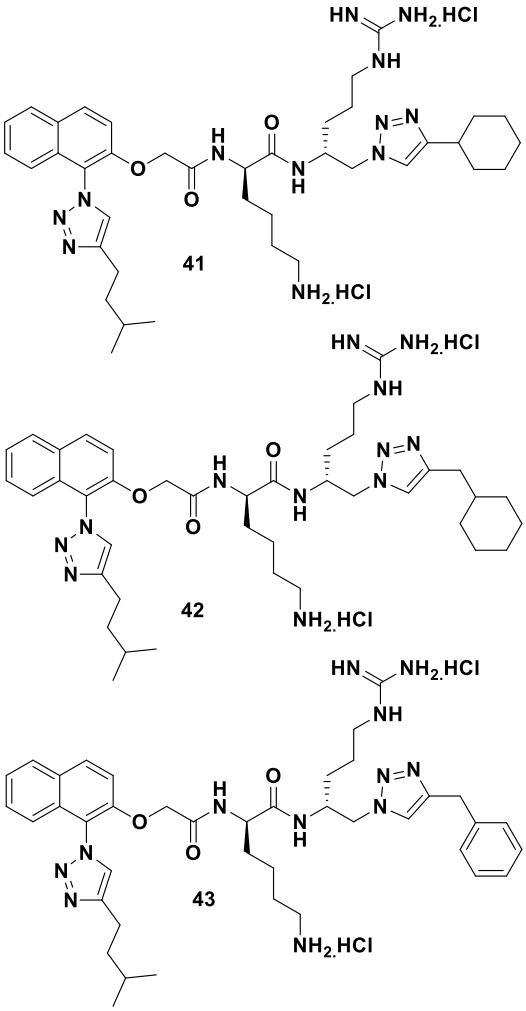
Table 3.8 – MIC or CC_{50} values for the various control inhibitors utilized in the secondary antimicrobial screening – reported in μg/mL.

3.2.3 –Overview of MIC assay results and lead compound identification

The newly synthesized compounds displayed antimicrobial activities in a range from active (MIC = 4 μg/mL) to inactive (MIC = >128 μg/mL) (Table 3.2 – 3.6 and 3.8). Many of the compounds showed an antibacterial activity against the Gram-positive bacteria rather than the Gram-negative bacteria; compounds generally being 2 – 4 times more potent against the Gram-positive strains. For example, compound **30** displayed an MIC value of 4 μg/mL against *S. aureus* but against *E. coli* the MIC value was 32 μg/mL (Table 3.3). Additionally, all the Gram-positive strains, including *C. difficile*, was less susceptible to the assessed compounds especially the hypervirulent *C. difficile* strain (RT027) (Tables 3.2 – 3.6). The additional membrane of Gram-negative bacteria is identified as an interruption for drug absorption.[13] MIC values in the range of 4 – 32 μg/mL were observed against the Gram-positive bacteria (Tables 3.2 – 3.6), whereas against the Gram-negative bacteria the MIC values are in the range of 32 – >128 μg/mL (Tables 3.2 – 3.6).

Lead compounds:

Compounds **41 – 43** exhibited the best antibacterial activities against both strains of *C. difficile* from the 29 derivatives tested in the primary and secondary screening. Compounds **41 – 43** also exhibited antimicrobial activity (MIC = 4 – 32 μg/mL) against Gram-positive bacteria. Compound **42** exhibited some activity (MIC = 16 μg/mL) against Gram-negative *P. aeruginosa*. Compounds **41 – 43** (Figure 3.2) exhibited antibacterial activities of 8 – 16 μg/mL against *C. difficile* in the primary screening. Compounds **41** and **43** showed low cytotoxicity (CC_{50} = 32 μg/mL and 32 μg/mL), however compound **42** exhibited some cytotoxicity (CC_{50} = 16 μg/mL) (Figure – 3.2). These studies concluded that either compounds **41** or **43** should be further tested in an *in vivo* murine model of CDI.



Bacterial Species	41	42	43	Vancomycin	Colistin
S. aureus	8	4	8	1	-
MRSA	16	8	8	1	-
E. faecalis	32	8	8	4	-
S. pneumoniae	16	16	8	1	-
C. difficile	8	16	8	0.5	-
C. difficile (RT027)	8	16	8	0.5	-
E. coli	64	128	32	-	0.125
P. aeruginosa	32	16	32	-	0.25
K. pneumoniae	>32	32	>32	-	0.25
A. baumannii	32	32	32	-	0.25
Cytotoxicity (CC_{50})	32	16	32		

Figure 3.2 – MIC and CC_{50} values (μg/mL) for lead compounds **41 – 43**.
(See Table 3.1 for the specific bacterial strains that were tested**)**

Compound **41** was chosen for an *in vivo* CDI mouse model study as it exhibited an MIC value of 8 μg/mL against the *C. difficile* in preliminary screening at University of Western Australia and follow-up screening against the hypervirulent *C. difficile* strain (M7404 – RT027) at Monash University disclosed the same MIC value of 8 μg/mL. Additionally, compound **41** exhibited only mild cytotoxicity (CC_{50} = 32 μg/mL; Table 3.8) and good water solubility (Table 3.9).

3.2.4 – Mechanism of action

MBC (minimum bactericidal concentration) screening of all synthesized compounds were performed at UWA. Several reports have earlier disclosed that 'membrane depolarization' was a possible mechanism of action for the antimicrobial potency of numerous cationic peptide compounds.[73-76] Other reports have specified that 'lysis of the cellular membrane' was another mechanism of action that causes the death of bacteria through enzyme mediated lysis which occurs after the drug causes the bacteria to form a defective cell wall.[73, 76] The MBC data was not included in the analysis as most of the compounds displayed MBC data that were almost equally related with their measured MIC data.

3.2.5 – HPLC purity assay

To confirm sample purity for the *in vivo* CDI murine model, compound **41** was subjected to reverse-phase HPLC analysis by using acetonitrile (non-polar) and H_2O (polar) (both with 0.1% v/v TFA) as solvents. A gradient elution from 0:100 → 100:0 (acetonitrile:H_2O) over 40 min was applied and the compound was eluted between 30 – 40 min. At 215 nm, HPLC analysis was confirmed the purity of the compound **41** was > 98%.

3.2.6 – Comparative solubility assay

The previously prepared binaphthyl compounds were generally insoluble in the 10% DMSO/H_2O solution which was used in the mouse model study of CDI. Hence, a comparative solubility assay was established to examine the solubility profile of the prepared compounds. This was useful to identify compounds that are expected to perform well in the

mouse model of CDI. Lead compound **1** (AVX-13616) was employed as a model compound for this assay and all other final derivatives were compared with this lead.

Five milligrams of lead compound **1** was dissolved in 50 μL of DMSO and then 5 μL H_2O aliquots were added with sufficient manual agitation in between H_2O additions. Addition of H_2O was continued until a precipitation or turbidity and cloudiness was apparent (which was did not disappear upon agitation). Compound **1** precipitated after the addition of 15 μL H_2O.

For example, if a test compound required 30 μL of water to produce precipitation or turbidity and cloudiness from its DMSO solution, then that test compound solubility was calculated as two times more soluble than compound **1** (i.e. solubility ratio – 2). This solubility ratio was used to calculate the relative solubility of the test compound allowing comparison between several similar compounds. The solubility assay was performed on eight compounds to determine their solubility ratio against the lead compound **1** (Table 3.9).

Solubility Assay Data

Compound	**H_2O ppt. vol** (µL)	**Solubility Ratio** (Compound : Compound-**1**)	**ClogP**
1	15	1	7.47
37	110	7.33	2.73
40	105	7	3.98
29	90	6	4.01
41	75	5	4.46
42	70	4.66	4.99
43	60	4	3.91
44	70	4.66	4.39
46	60	4	3.93

Table 3.9 – Comparative solubility assay data and CLogP calculated using (Chemdraw 16.0) of compounds.

The CLogP data (calculated using Chemdraw 16.0) for the tested compounds are also added to the solubility table (Table – 3.9). The ClogP data confirmed that the tested compounds showed ClogP values in the range of 2.73 – 4.99 due to the replacement of the hydrophobic binaphthyl ring in compound **1** (ClogP 7.47) with the more polar phenyl-1,2,3-trizole or naphthyl-1,2,3-triazole group. The phenyltriazole compound **37** showed a ClogP value of 2.73, lower than that of the naphthyltriazole compound **42** (Table – 3.9) which showed a ClogP value of 4.99 due to the more hydrophobic naphthyl ring system. This result

was as expected with the larger hydrophobic groups decreasing the solubility of the compound in water.

As expected, the dicationic phenyltriazole compound **37** was more soluble than its related naphthyl analogue **43** (Table 3.9). This impact is further illustrated by the dicationic naphthalenetriazole derivative **46** and the subsequent phenyltriazole analogue **40** which is almost two times more soluble (Table 3.9). Moreover, altering the position of the isopentyl group from the C – 4 position to the C – 5 position does not have an impact on solubility; for example, compound **37** (isopentyl group at the C – 4 position on the triazole) has the same solubility as the corresponding isomer **40** (isopentyl group at C – 5 position on the triazole). This effect is further illustrated by the compounds **43** and **46** which both have the same solubilities (Table 3.9). Furthermore, a flexible hydrocarbon termini (i.e. Cy) was usually more soluble than an aromatic termini i.e. Bn.

3.3 – *In vivo* assay: murine model of CDI

3.3.1 – General methodology

The murine model of CDI was conducted with project collaborators at Monash University. The murine model of CDI was performed by pretreating cohorts of five mice that had been earlier infected *via* oral gavage with hypervirulent M7404 *C. difficile* spores. The infection was allowed to develop for 12 h before the trial drug was administered every 12 h for five days. The mice received a single dosage of 2.5 mg of compound **41** (i.e. 100 mg/kg for an average 25 g mouse) administered in a solvent mixture of 10% DMSO and H_2O at each 12 h dosing period. The mice body weight was checked every day. If a mouse lost 10% of its body weight in the first 24 h, then the infection was developing, and the mouse was separated from the trial and culled for ethical reasons. The whole survival rate for every drug

cohort was utilized to measure effectiveness of that drug. Other physiological parameters such as cage appearance scores and faecal consistency scores were additional measures for the efficacy of the drug in this murine trial. The experimental data of the *in vivo* CDI model can be found in Section 3.3.3 and the experimental procedures can be found in Section 6.4.3.

3.3.2 – Preliminary trials

Compound **41** was selected for a murine model of CDI study because of its better antimicrobial potency against *C. difficile* and its better water solubility profile (Section 3.2.7). Compound **41** was prepared on a larger scale i.e. nearly 300 mg for the purpose of *in vivo* mouse model of CDI at Monash University. In the first trial, attempts were made to administer compound **41** through drinking water, but the mice refused to drink the adulterated water. As a result, the mice were dehydrated and lost weight due the effects of the infection.

3.3.3 – Secondary trial

In the secondary CDI mouse model trial, the solution (10% v/v DMSO/H_2O) of the compound **41** was administered to mice by oral gavage.

The mice that were treated with compound **41** (orange line in Figure 3.3) showed 80% survival rate until 24 h, then continued at the same survival rate for another 6 h. After 42 h, the survival rate dropped to 60 – 65% (compared with a 40% survival rate in the DMSO control cage group).

Kaplan-Meier survival curve:

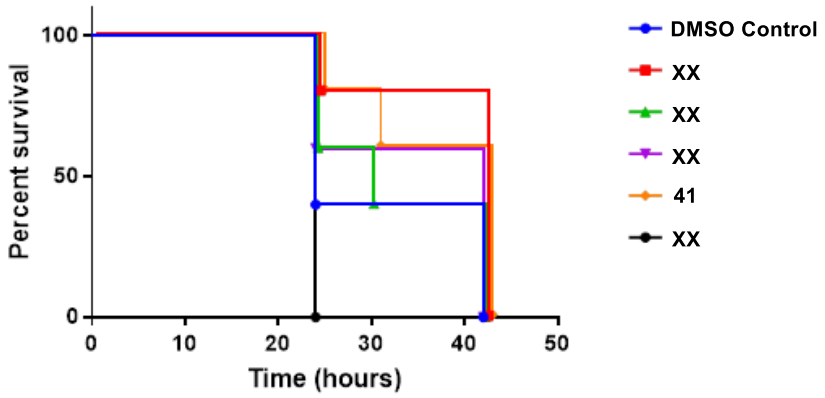


Figure 3.3 – Kaplan-Meier survival curve for the *in vivo* CDI mouse trial of compound – **41** (Orange). XX refer to other compounds, not described in this thesis, that were tested concurrently with **41**.

The mice that were treated with compound **41** lost the least amount of weight at 24 h (Figure 3.4) and after 42 h, the mice lost 18% weight.

Weight loss percentage:

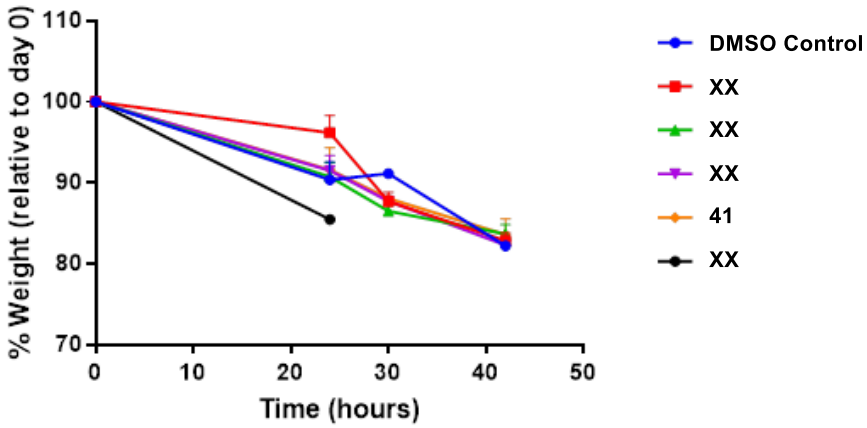


Figure 3.4 – Percentage weight loss for the compound – **41** (Orange) cohort in the *in vivo* CDI mouse model. XX refer to other compounds, not described in this thesis, that were tested concurrently with **41**.

The mice that were treated with compound **41** had moderately soft stools, that appeared very moist and sign of soiling around the anus based on the score from faecal consistency (Figures 3.5).

Faecal consistency:

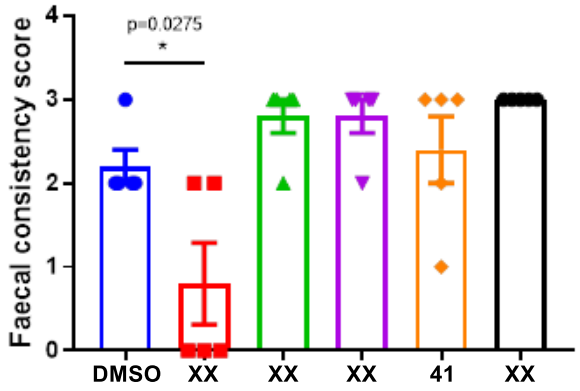


Figure 3.5 – Faecal consistency scores for the compound – **41** (Orange) cohort in the *in vivo* CDI mouse model. XX refer to other compounds, not described in this thesis, that were tested concurrently with **41**.

Stool scoring system:

(0) **Normal stool:** Solid stool that is firm when subjected to pressure with forceps. Mouse has no sign of soiling around the anus and passes the stool quickly and easily.

(1) **Mildly soft stool:** Formed stools that appear moist on the outside and have a slightly sticky consistency. Stools will easily submit to pressure applied with forceps. Mouse has no sign of soiling around the anus but passes faeces quickly and easily.

(2) **Moderately soft stool:** Irregularly formed stools that do not hold a normal shape. Stool appears very moist and is difficult to pick up with forceps. Mouse has some signs of soiling around anus and takes a longer than normal time to pass the stool.

(3) **Diarrhoea:** Stool has no form and/or has a mucous-like liquid appearance with minimal solid present. Considerable soiling around the anus and the fur around tail. Mouse takes a long time to pass stool if at all.

The mice given compound **41** had a lower cage appearance score when compared to the DMSO controls which suggested that this compound was delaying diarrhoea (Figures 3.6).

Cage condition:

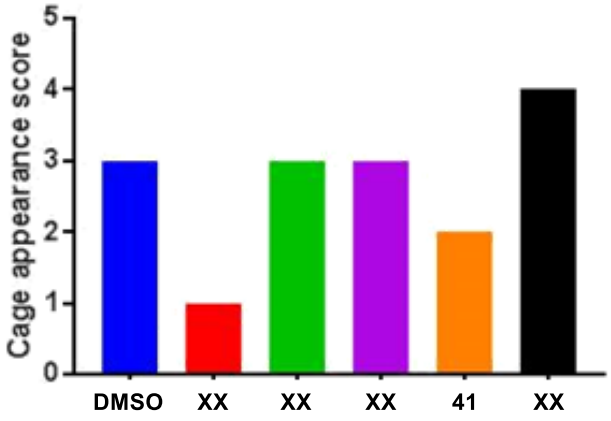


Figure 3.6 – Cage condition score for the compound – **41** cohort in the *in vivo* CDI mouse model. XX refer to other compounds, not described in this thesis, that were tested concurrently with **41**.

Cage appearance scoring system:

0 – Normal cage.

1 – Faeces stuck to the side of cage, but clean nest and saw dust.

2 – Mildly soiled nest.

3 – Moderately soiled nest.

4 – Severely soiled nest.

The mice appeared to be physically changed based on the score of physiological appearance as they showed signs of reduced grooming with their coats starting to appear rough/scruffy with slight piloerection (Figures 3.7).

Physiological appearance:

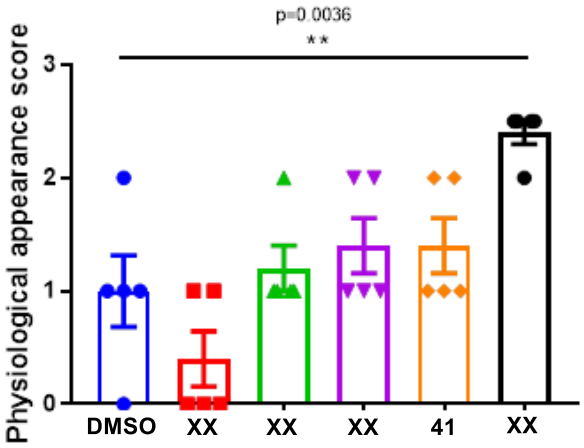


Figure 3.7 – Physiological appearance score for the compound – **41** cohort in the *in vivo* CDI mouse model. XX refer to other compounds, not described in this thesis, that were tested concurrently with **41**.

Physiological appearance scoring system:

0 – Normal activity, alertness, breathing and movement/gait.

1 – Mouse shows signs of reduced grooming with coat starting to appear rough/scruffy with slight piloerection.

2 – Mouse appears slightly hunched with mildly scruffy coat but moves when the cage is disturbed. Possible diminished alertness, squinted eyes or lethargy.

3 – Mouse has isolated itself from cage mates, displays hunched posture and unkempt coat. Mouse does not move when cage is disrupted and has little response to external environment or handling. Possible laboured breathing or eye discharge.

3.3.4 – Conclusions from murine model of CDI study

In the initial murine model of CDI study, attempts were made to administer compound **41** through the drinking water, but the mice refused to drink the adulterated water. Therefore, the mice were dehydrated, followed by lost weight because of the infection.

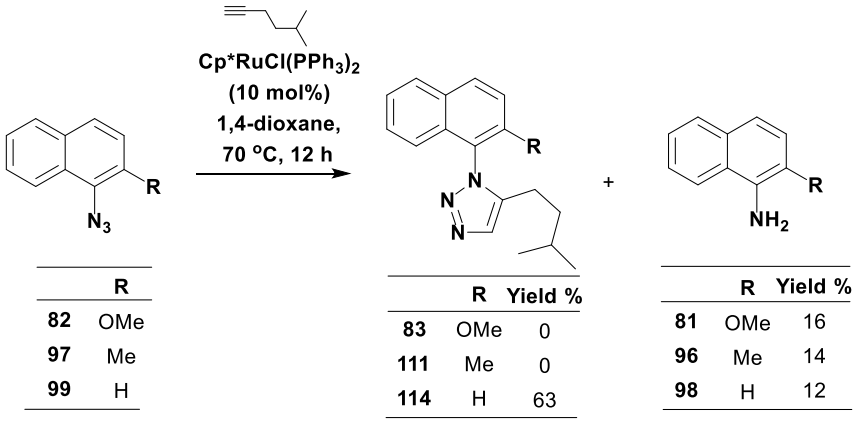
In the secondary trial of this study, compound **41** was administered *via* oral gavage. At 24 hours post-infection, compound **41** appeared to be protecting the mice from disease.

The mice given compound **41** lost the least amount of weight at 24 hours and had the mild diarrhoea (as shown by the faecal consistency score and cage appearance score). Furthermore, the mice had a lower cage appearance score when compared to the DMSO controls, (suggesting that this compound was also delaying diarrhoea) and also mice had survived until 42 hours. Compound **41** displayed some promising activity, despite not completely protecting the mice in the murine model of CDI study and the mice had to be culled after 42 h due to weight loss from the disease (Figure 3.4).

Compound **43** also displayed similar *in vitro* antimicrobial potency as compound **41** (MIC = 8.0 µg/mL) against hypervirulent *C. difficile* with better water solubility, indicating that this compound should be prepared on a larger scale for future *in vivo* mouse model studies. Further drug developments will likely focus on structural modifications of the *N*-naphthyltriazole series. These modifications could include switching the positions of naphthalene group and triazole ring. These modifications might produce novel peptidomimetics with increasing antibacterial activity and good water solubility; which is an essential requirement for the future development of this project.

4.0 – Click methodology: results and discussion

In Chapter 2, it was noted that the attempted synthesis of 5-isopentyl-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole **83** from 1-azido-2-methoxynaphthalene **82** using Cp*RuCl(PPh$_3$)$_2$ in 1,4-dioxane at 70 °C for 12 h (Chapter 2; Section – 2.1.1.4; Scheme 2.7), resulted in the formation of 1-amino-2-methoxynaphthalene **81** in 16% yield. None of the desired 1,5-triazole product **83** was formed. It was suspected that the 0.2 equivalents of PPh$_3$ from the ruthenium catalyst resulted in the formation of the amine **81** *via* a Staudinger reaction of the azide group of **82**. To confirm the involvement of PPh$_3$, when the azide **82** was treated with Cp*RuCl(PPh$_3$)$_2$ in the absence of 5-methyl-1-hexyne at 70 °C in 1,4-dioxane the amine **81** was also produced in 16% yield (Scheme 4.1). The steric bulk of the arylazide and the Cp*RuCl(PPh$_3$)$_2$ catalyst were most likely the reason for the lack of formation of the 1,5-triazole **83**.



	R
82	OMe
97	Me
99	H

	R	Yield %
83	OMe	0
111	Me	0
114	H	63

	R	Yield %
81	OMe	16
96	Me	14
98	H	12

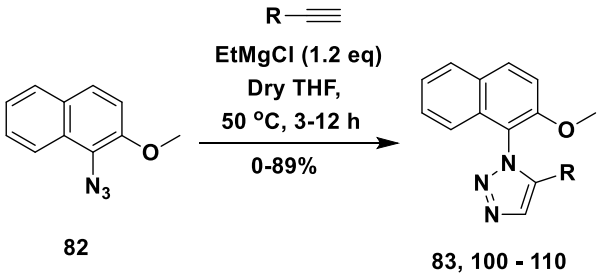
Scheme 4.1 – Attempted synthesis of the click products **83**, **111** and **114** from azides **82**, **97** and **99**

Consistent with this premise was the fact that the reaction of azide **97** (R = Me) with 5-methyl-1-hexyne in the presence of Cp*RuCl(PPh$_3$)$_2$ at 70 °C for 12 h resulted in formation of the amine **96** in 14% yield and none of the desired triazole **111** was formed (Scheme 4.1). In contrast, the less hindered azide **99** produced the desired cycloaddition product **114** in 63% yield and amine **98** in 12% yield, when treated with 5-methyl-1-hexyne in the presence of

$Cp^*RuCl(PPh_3)_2$ at 70 °C for 12 h (Scheme 4.1). These results suggest that the more hindered azides **82** and **97** are undergoing a Staudinger reaction with the PPh_3 derived from the Ru-catalyst rather than undergoing the desired cycloaddition reaction. These results initiated our study using as an alternative method, the Mg-promoted click reaction conditions developed by Sharpless *et al*.[92] We assumed that this would result in a less sterically demanding reactive intermediate and would be more fruitful. This chapter reports the outcomes of a study of the cycloaddition reactions of hindered naphthyl azides and alkynes.

4.1 – Synthesis of 1,5-triazoles *via* magnesium-promoted cycloaddition reactions

The results of the study of the magnesium-promoted cycloaddition reactions of the azides **82**, **97** and **99** with a variety of relatively hindered, electron rich and electron deficient alkynes are shown in Schemes 4.2 – 4.4. The results of the reactions with azide **82** are shown in Scheme 4.2. The alkynes were first treated with EtMgCl (1.2 equivalents) in dry THF at 50 °C for 30 min to generate the corresponding chloromagnesium acetylide. A solution of the azide in dry THF was then added slowly dropwise to the acetylide at rt and the mixture was then heated at 50 °C for 3 – 12 h.



82 → **83, 100 - 110**

	R	Time (h)	Yield %
83	^{i}Pent	3	81
100	*c*-C_5H_{11}	5	78
101	4-MeO-Ph	3	86
102	2-MeO-Ph	4	89
103	2-Me-Ph	3	76
104	3-F-Ph	5	86
105	2,4,6-triMe-Ph	6	52
106	2,6-diMeO-Ph	12	0
107	4-F-Ph	12	0
108	4-NO_2-Ph	12	0
109	3-NO_2-Ph	12	0
110	1-naphthyl	12	0

Scheme 4.2 – Synthesis of the cycloaddition products **83** and **100 – 110** from azide **82**

The reaction between azide **82** and chloromagnesium-5-methyl-1-hexylide gave the 1,5-triazole **83** (Scheme 4.2) in 81% yield. The ^{1}H NMR spectrum of **83** displayed the characteristic resonances of the naphthalene group as a doublet resonance at δ_H 8.03 (J = 9.1 Hz, 1H), a doublet of doublet resonance at δ_H 7.85 (J = 7.4, 2.0 Hz, 1H) and as multiplet resonances at δ_H 7.45 – 7.37 (3H) and δ_H 6.99 – 6.96 (1H) (Figure 4.1). The characteristic singlet resonance at δ_H 3.87 was assigned for the OMe group (Figure 4.1). The characteristic resonances (11H) that were assigned to the isopentyl group appeared as multiplet resonances at δ_H 2.45 – 2.34 (2H), δ_H 1.47 – 1.35 (3H) and the two doublet resonances at δ_H 0.74 (J = 7.8 Hz, 3H) and δ_H 0.71 (J = 7.8 Hz, 3H) (Figure 4.1). The characteristic singlet resonance at δ_H 7.71 was assigned to the triazole ring proton H-4 (Figure 4.1).

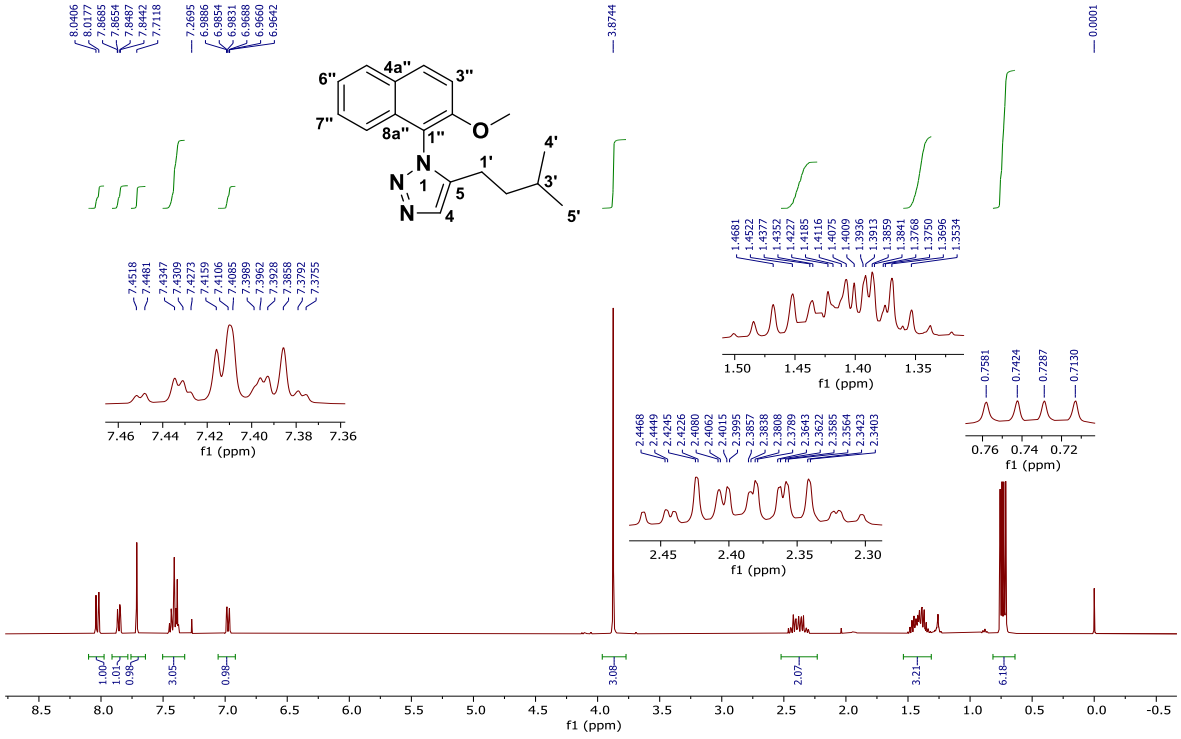


Figure 4.1: ^{1}H NMR spectrum of compound **83** (400 MHz, $CDCl_3$)

The C-4 carbon of the triazole ring resonated at δ_C 131.6 and was assigned from the gHSQC spectrum (Figure 4.2).

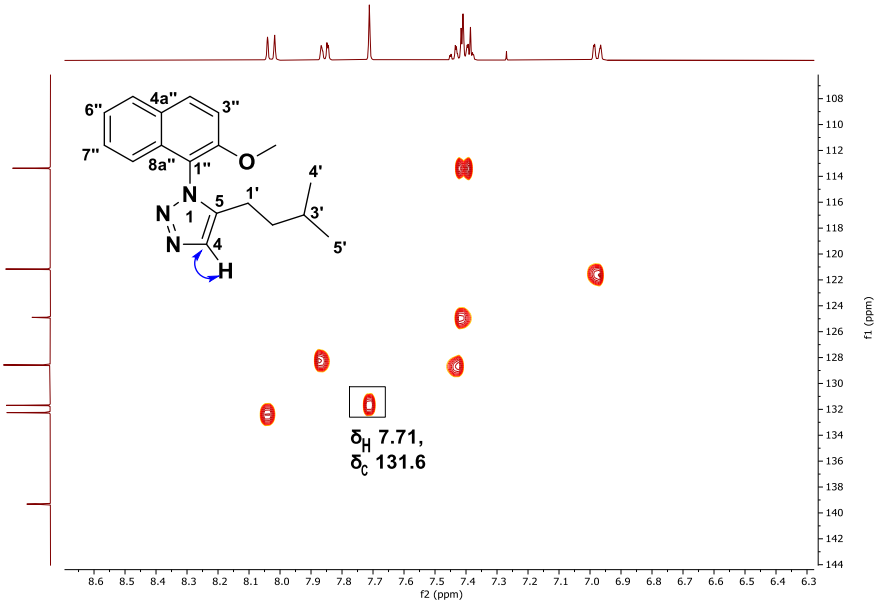


Figure 4.2: gHSQC spectrum of compound **83** (500 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-4 is highlighted

The gHMBC spectrum of **83** allowed the assignment of the resonance at δ_C 140.7 to the quaternary C-5 carbon of the triazole ring (Figure 4.3).

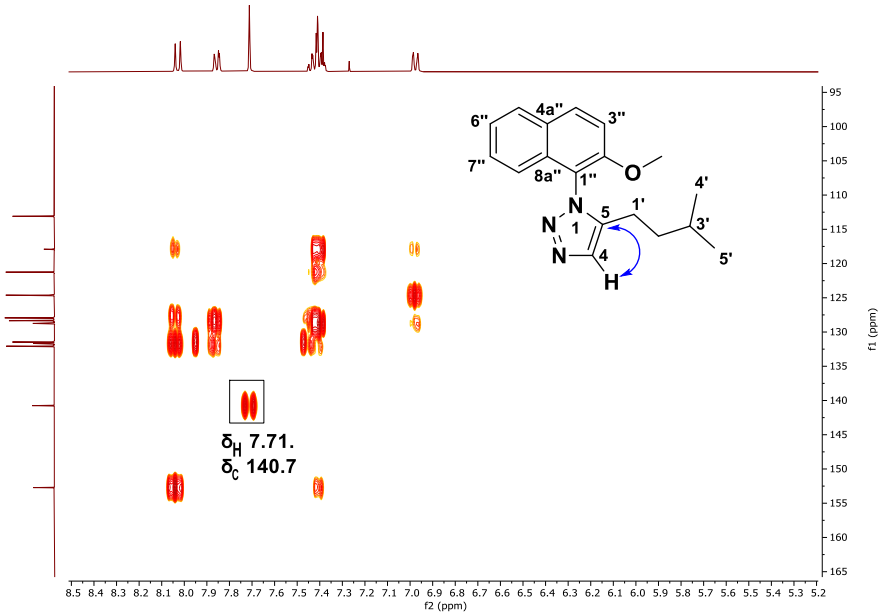
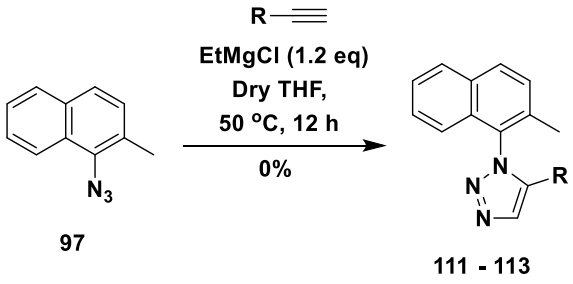


Figure 4.3: gHMBC spectrum of compound **83** (500 MHz, $CDCl_3$). The two-bond correlation between the triazole proton and C-5 is highlighted

Furthermore, the triazole proton did not show a correlation with the naphthalene C – N carbon (C1") in the gHMBC spectrum. This was consistent with **83** having the expected 1,5-disubstituted triazole structure (Scheme 4.2), whereas its regioisomers **123** (Figure 4.13) showed a correlation between the naphthalene C1" and the C5 triazole proton, indicating the presence of the triazole-1,4-regioisomer.. The molecular structure of compound **83** was further established by the presence of the ion peak at *m/z* 296.1750 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{18}H_{22}N_3O$ 296.1763).

The 1,5-triazoles **100** – **105** were also successfully prepared from azide **82** in yields ranging from 52 – 89% (Scheme 4.2). The reactions involving 3-fluorophenylacetylene and 2,4,6-trimethylphenylacetylene required extended reaction times (5 h and 6 h, respectively) presumably due to electronic effects (strongly electron withdrawing) and steric effects (2 *ortho* Me groups), respectively. The reaction of azide **82** and 3-fluorophenylacetylene gave the desired 1,5-triazole **104** (Scheme 4.2) in 86% yield, but the synthesis of **107** from the reaction of azide **82** with 4-fluorophenylacetylene was unsuccessful possibly due to the more electron-withdrawing effect from fluorine substitution at the *para* position of the arylalkyne. The synthesis of the 1,5-triazoles **106** – **110** (Scheme 4.2) from azide **82** was unsuccessful due to the steric hinderance (**106** and **110**) and electron-withdrawing properties (**107** – **109**) of the corresponding alkynes. The reason for the unsuccessful synthesis of **106** is not clear since sterically this product should be more favored than that of **105**. In the case of 3- and 4-nitrophenylacetylenes, the addition of EtMgCl resulted in a black solution indicating an undesired reaction between EtMgCl and the alkyne, possible due to an electron transfer reaction to the NO_2 group.

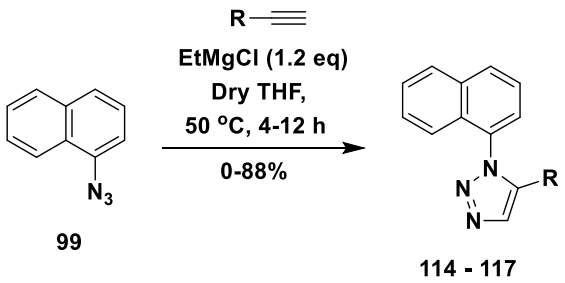
Under similar reaction conditions to those used for **82**, the reactions of the azide **97** (Scheme 4.3) with alkynes (R = isopentyl, 2-MeO-Ph and 1-naphthyl) were not successful due to the more sterically hindered nature of the azide **97** (2-Me substituent is more sterically demanding than the 2-OMe group of azide **82**).



	R	Time (h)	Yield %
111	^{i}Pent	12	0
112	2-MeO-Ph	12	0
113	1-naphthyl	12	0

Scheme 4.3 – Attempted synthesis of the cycloaddition products **111 – 113** from azide **97**

The less sterically demanding naphthyl azide **99** gave the 1,5-triazoles **114** – **116** in yields of 10 – 88%. The reaction of the azide **99** with more hindered 2-MeO-phenylacetylene giving a low yield of 10%, after heating at 50 °C for 12 h (Scheme 4.4). The synthesis of the 1,5-triazole **117** was unsuccessful due to the more sterically hindered nature of the alkyne (i.e. 1-ethynylnaphthalene).



	R	Time (h)	Yield %
114	^{i}Pent	4	88
115	*c*-C_5H_{11}	4	88
116	2-MeO-Ph	12	10
117	1-naphthyl	12	0

Scheme 4.4 – Synthesis of the cycloaddition products **114 – 117** from azide **99**

The reaction between azide **99** and chloromagnesium-5-methyl-1-hexylide gave the 1,5-triazole **114** (Scheme 4.4) in 88% yield. The ^{1}H NMR spectrum of the 1,5-triazole **114**

displayed characteristic resonances for the naphthalene group i.e. δ_H 8.04 (d, J = 8.3 Hz, 1H), δ_H 7.95 (d, J = 8.2 Hz, 1H), δ_H 7.61 – 7.54 (m, 2H), δ_H 7.50 – 7.47 (m, 2H) and δ_H 7.16 (dd, J = 8.5, 0.8 Hz, 1H) (Figure 4.4). The isopentyl group showed resonances at δ_H 2.45 – 2.42 (m, 2H), δ_H 1.46 – 1.36 (m, 3H) and δ_H 0.71 (d, J = 6.2 Hz, 6H) (Figure 4.4). The singlet resonance at δ_H 7.70 was assigned for the triazole ring proton H-4 (Figure 4.4).

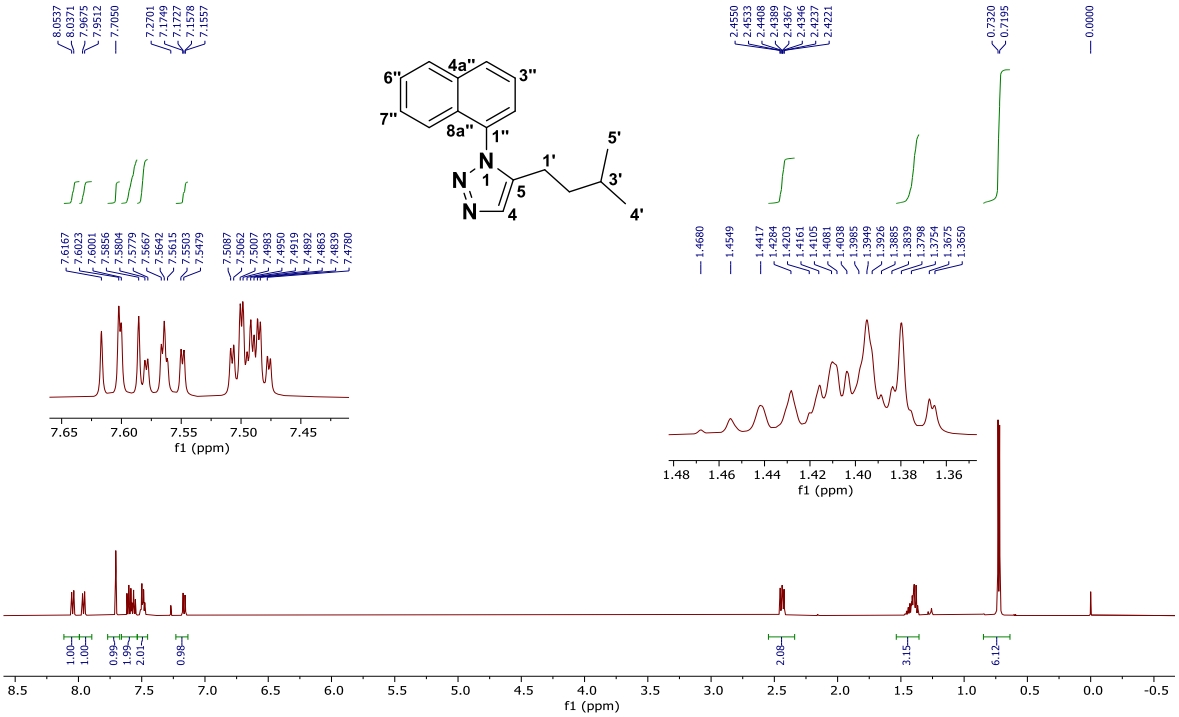


Figure 4.4: ^{1}H NMR spectrum of compound **114** (500 MHz, $CDCl_3$)

The gHSQC spectrum allowed the resonance at δ_C 131.8 to be assigned to C-4 (Figure 4.5).

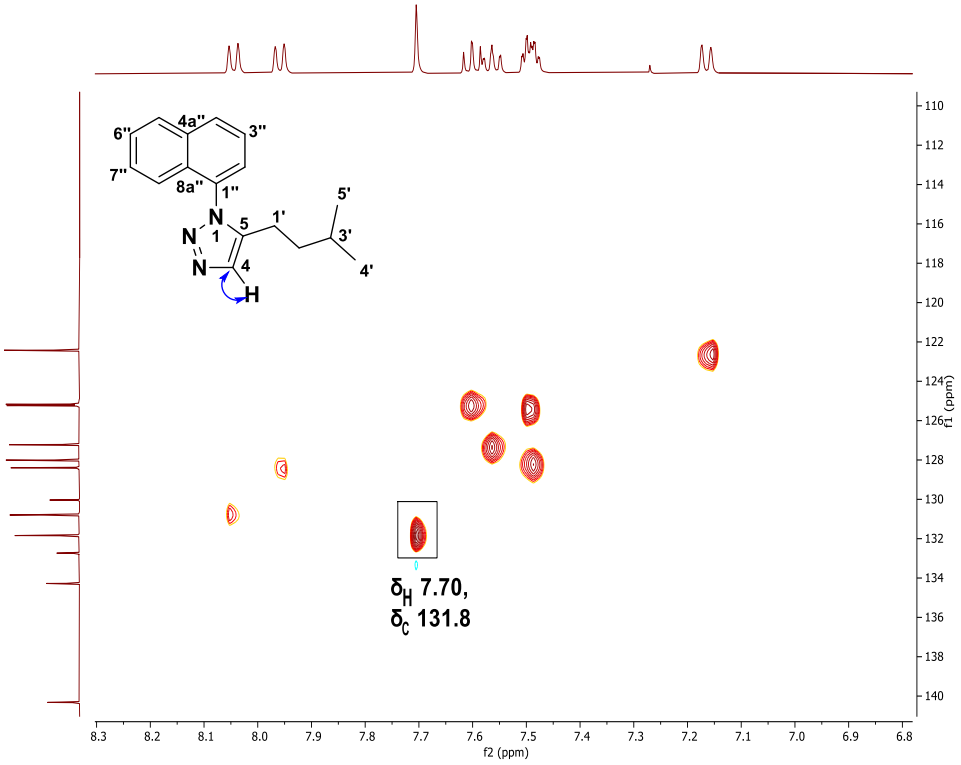


Figure 4.5: gHSQC spectrum of compound **114** (500 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-4 is highlighted

The gHMBC spectrum allowed the resonance at δ_C 140.3 to be assigned to quaternary C-5 of the triazole (Figure 4.6).

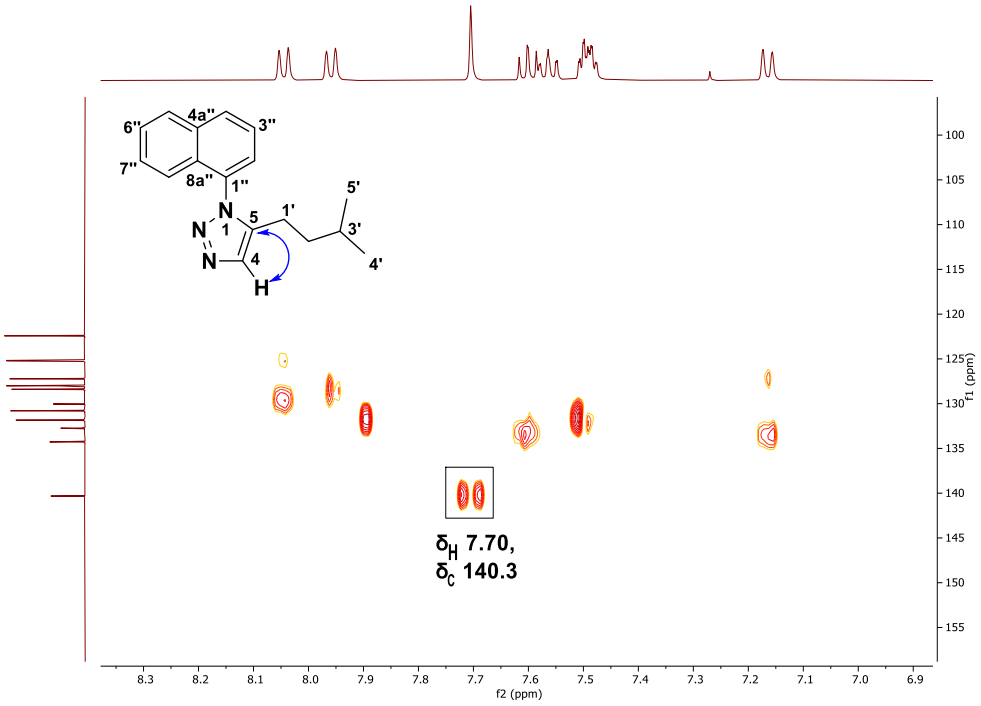


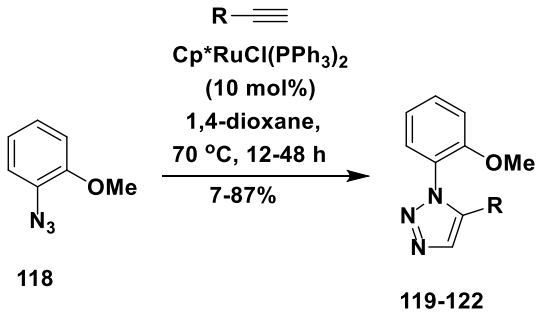
Figure 4.6: gHMBC spectrum of compound **114** (500 MHz, $CDCl_3$). The two-bond correlation between the triazole proton and C-5 is highlighted

The triazole proton did not show a correlation with the naphthalene C – N carbon (C1"), which was consistent with the 1,5-disubstituted triazole structure of **114** (Scheme – 4.4). The molecular structure of compound **114** was further confirmed by the presence of the ion peak at *m/z* 266.1668 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{17}H_{20}N_3$ 266.1657).

The synthesis of the 1,5-triazole **117** (Scheme – 4.4) from the azide **99** was unsuccessful due to the highly sterically hindered nature of the alkyne (i.e., 1-ethynylnaphthalene). The mechanism of Mg-promoted cycloaddition reaction was discussed in section – 2.1.1.4; Scheme – 2.9 of Chapter-2.

4.2 – Synthesis of 1,5-disubstituted-1,2,3-triazoles using pentamethylcyclopentadienyl bis(triphenylphosphine)ruthenium(II) chloride

A study of the synthesis of phenyl 1,5-triazoles **119 – 122** was also undertaken. The azide **118** was treated with four different alkynes using Cp*RuCl(PPh_3)$_2$ (10 mol%) as the catalyst in 1,4-dioxane at 70 °C for 12 – 48 h. The results are shown in Scheme - 4.5.



	R	Time (h)	Yield %
119	^{i}Pent	12	87
120	*c*-C_5H_{11}	12	78
121	2-MeO-Ph	36	58
122	1-naphthyl	48	7

Scheme 4.5 – Synthesis of the cycloaddition products **119 – 122** from azide **118**

The reaction of azide **118** with 5-methyl-1-hexyne gave the 1,5-triazole **119** in 87% yield. The ^{1}H NMR spectrum of the 1,5-triazole **119** displayed the characteristic resonances of the phenyl group as a multiplet resonance at δ_H 7.50 – 7.47 (1H), a doublet of doublet resonance at δ_H 7.32 (*J* = 7.9, 1.7 Hz, 1H) and as multiplet resonance at δ_H 7.11 – 7.06 (2H) (Figure 4.7). The characteristic singlet resonance at δ_H 3.78 was assigned to the OMe group. The characteristic resonances of the isopentyl group were observed at δ_H 2.47 (t, *J* = 7.9 Hz, 2H), δ_H 1.56 – 1.41 (m, 3H) and δ_H 0.82 (d, *J* = 8.0 Hz, 6H) (Figure 4.7). The characteristic singlet resonance at δ_H 7.55 was assigned to the triazole ring proton H-4 (Figure 4.7).

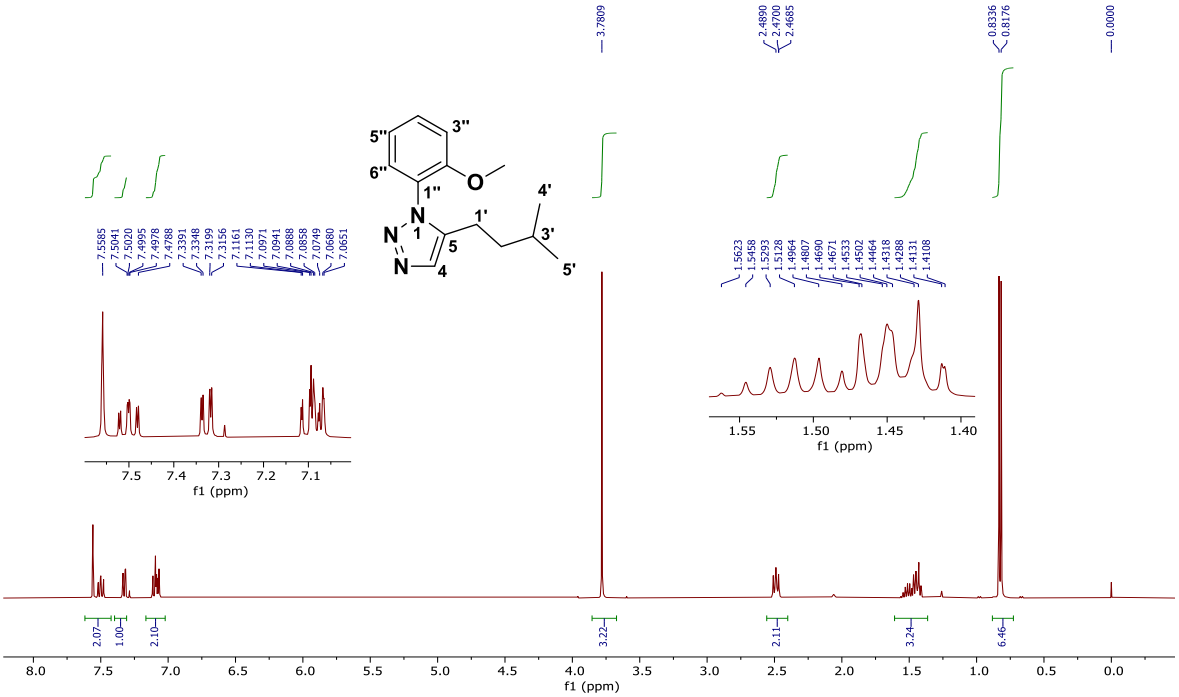


Figure 4.7: ^{1}H NMR spectrum of compound **119** (500 MHz, $CDCl_3$)

The C-4 carbon of the triazole ring assigned at δ_C 131.3 was assigned from the gHSQC spectrum (Figure 4.8).

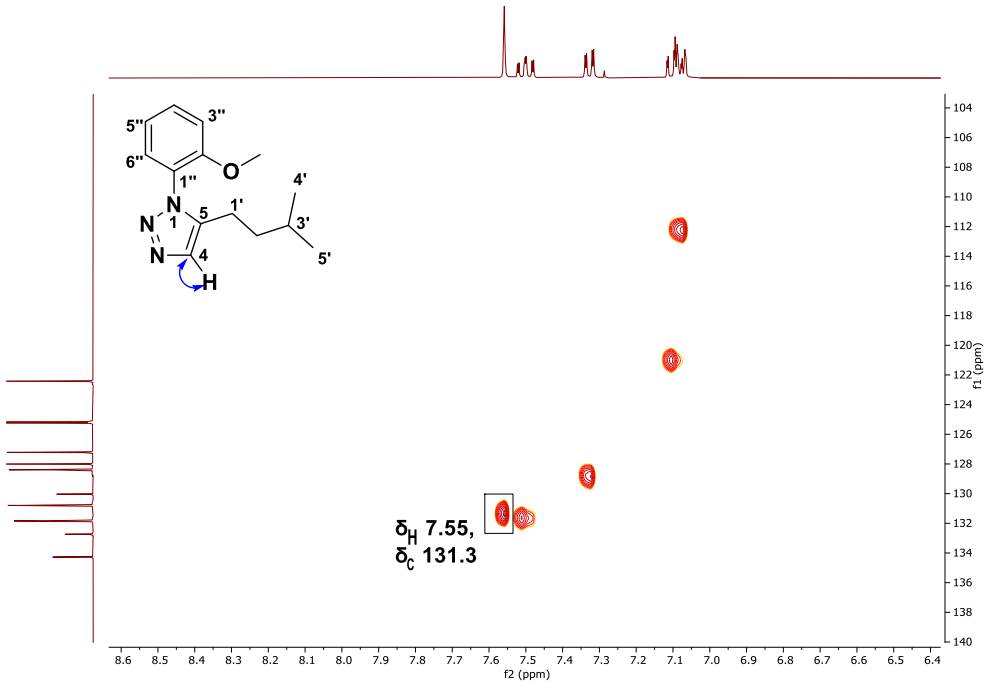


Figure 4.8: gHSQC spectrum of compound **119** (500 MHz, $CDCl_3$)**.** The one bond correlation between the triazole proton and C-4 is highlighted

The gHMBC spectrum of **119** allowed the assignment of the resonance at δ_C 140.0 to the quaternary C-5 carbon of the triazole ring (Figure 4.9).

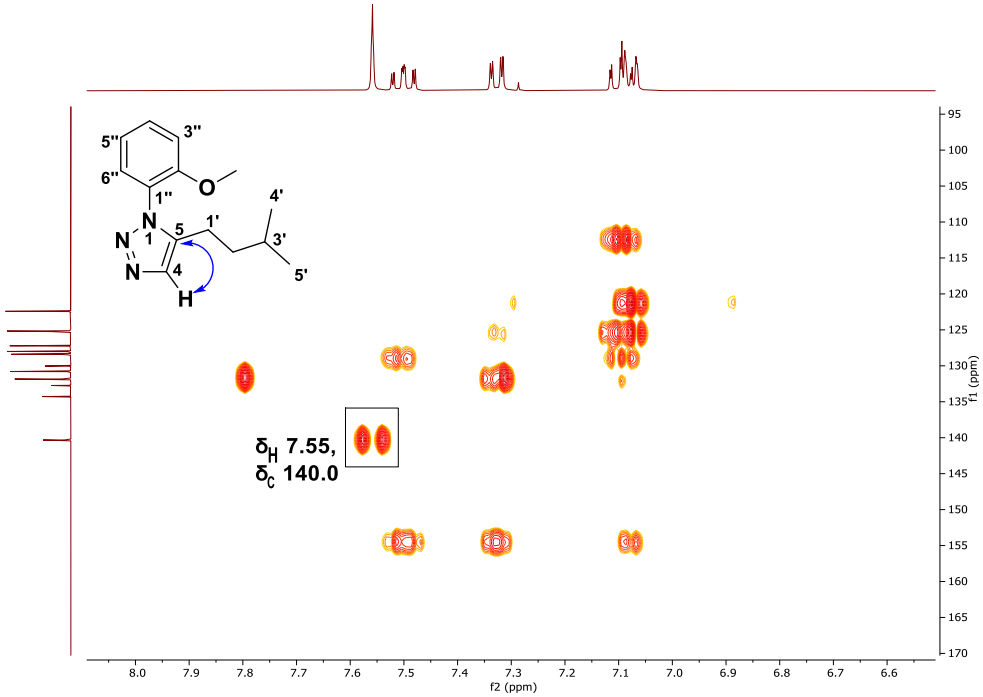


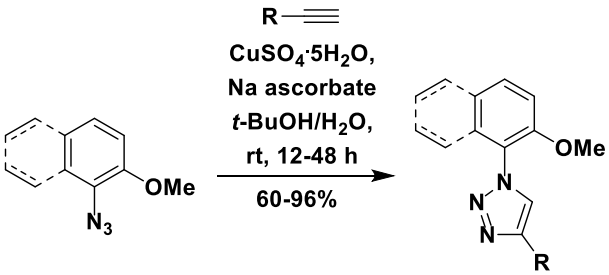
Figure 4.9: gHMBC spectrum of compound **119** (500 MHz, $CDCl_3$)**.** The two-bond correlation between the triazole proton and C-5 is highlighted

The triazole proton did not show a correlation with the phenyl C – N carbon (C1"); which was consistent with the 1,5-disubstituted triazole structures of **120 – 122** (Scheme – 4.5). The molecular structure of compound **119** was further verified by the presence of the ion peak at *m/z* 246.1618 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{14}H_{20}N_3O$ 246.1606).

The longer reaction time and the lower yield of **121** (58%) was attributed due to the more sterically hindered nature of the alkyne. The low yield of **122** (7%) was attributed to the highly sterically hindered nature of the alkyne (Scheme – 4.5). The mechanism of the Ru-catalyzed cycloaddition reaction was discussed in section – 2.1.1.3; Scheme – 2.5 of Chapter-2.

4.3 – Synthesis of 1,4-disubstituted-1,2, 3-triazoles from CuAAC reaction

In this study, the CuAAC reactions of the azides **82** and **118** with a variety of relatively sterically hindered alkynes were also examined (Scheme – 4.6). In the case of azide **82**, the reaction yields were high (79 – 92%) including those for hindered alkynes (R = 2-MeO-Ph, 1-naphthyl and CMe_2OH) and relatively electron deficient alkynes (4-F-Ph and 4-NO_2-Ph).



82: Naphthalene
118: Phenyl

123-132

		R	Time (h)	Yield %
123	Naph	^{i}Pent	36	85
124	Naph	*c*-C_5H_{11}	48	81
125	Naph	2-MeO-Ph	36	89
126	Naph	4-F-Ph	12	92
127	Naph	4-NO_2-Ph	16	79
128	Naph	1-naphthyl	12	91
129	Naph	CMe_2OH	12	86
130	Ph	^{i}Pent	12	96
131	Ph	*c*-C_5H_{11}	12	60
132	Ph	2-MeO-Ph	12	88

Scheme 4.6 – Synthesis of the cycloaddition products **123 – 132** from azides **82** and **118**

The reaction of azide **82** with 5-methyl-1-hexyne gave the 1,4-triazole **123** in 85% yield. The ^{1}H NMR spectrum of the triazole product **123** displayed characteristic resonances for the naphthalene group i.e., δ_H 7.98 (d, J = 11.4 Hz, 1H), δ_H 7.83 (d, J = 9.5 Hz, 1H), δ_H 7.46 – 7.35 (m, 3H) and δ_H 7.16 (dd, J = 10.5, 0.9 Hz, 1H) (Figure 4.10). The characteristic singlet resonance at δ_H 3.87 was assigned to the OMe group. The isopentyl group showed resonances at δ_H 2.91 – 2.87 (m, 2H), δ_H 1.73 – 1.69 (m, 3H) and δ_H 0.99 (d, J = 7.9, 6H) (Figure 4.10). The characteristic singlet resonance at δ_H 7.52 was assigned to the triazole ring proton (Figure 4.10).

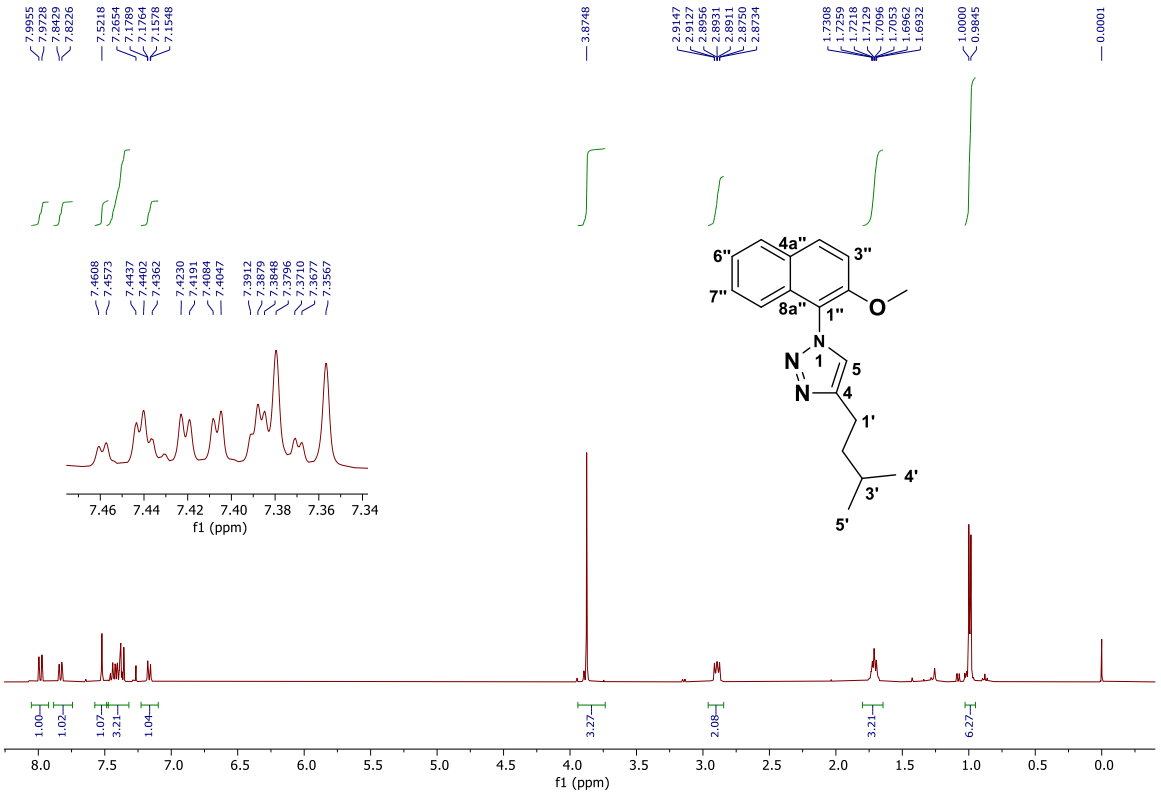


Figure 4.10: ^{1}H NMR spectrum of compound **123** (500 MHz, $CDCl_3$)

The gHSQC spectrum allowed the resonance at δ_C 124.3 to be assigned to C-5 (Figure 4.11).

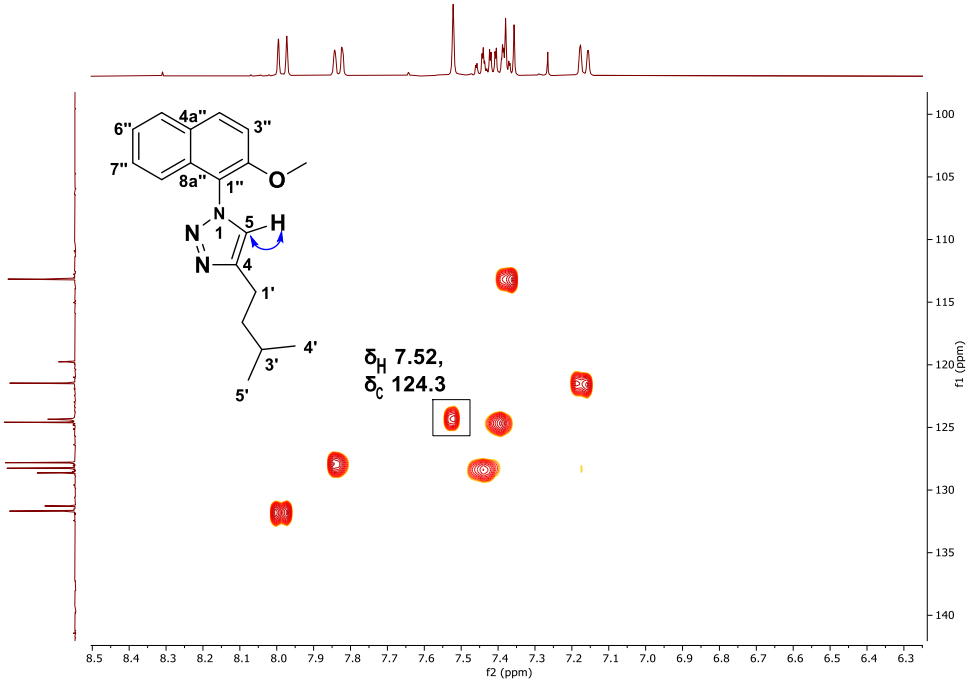


Figure 4.11 gHSQC spectrum of compound **123** (500 MHz, $CDCl_3$). The one bond correlation between the triazole proton and C-5 is highlighted

The gHMBC spectrum allowed the resonance at δ_C 148.1 to be assigned to quaternary C-4 of the triazole (Figure 4.12).

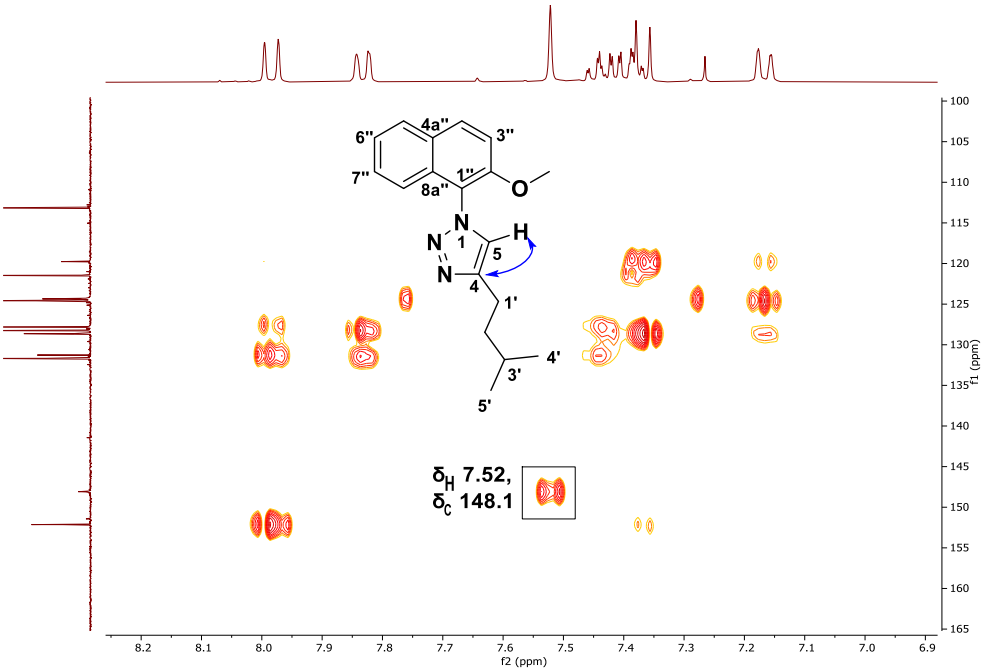


Figure 4.12: gHMBC spectrum of compound **123** (500 MHz, $CDCl_3$). The two-bond correlation between the triazole proton and C-4 is highlighted

Unlike its 1,5-triazole regioisomer **83**, the triazole proton of 1,4-triazole **123** showed a correlation with the naphthalene C – N carbon at δ_C 113.1 (C1") (Figure 4.13); which was consistent with the 1,4-disubstituted triazole structure of **123** (Scheme – 4.6).

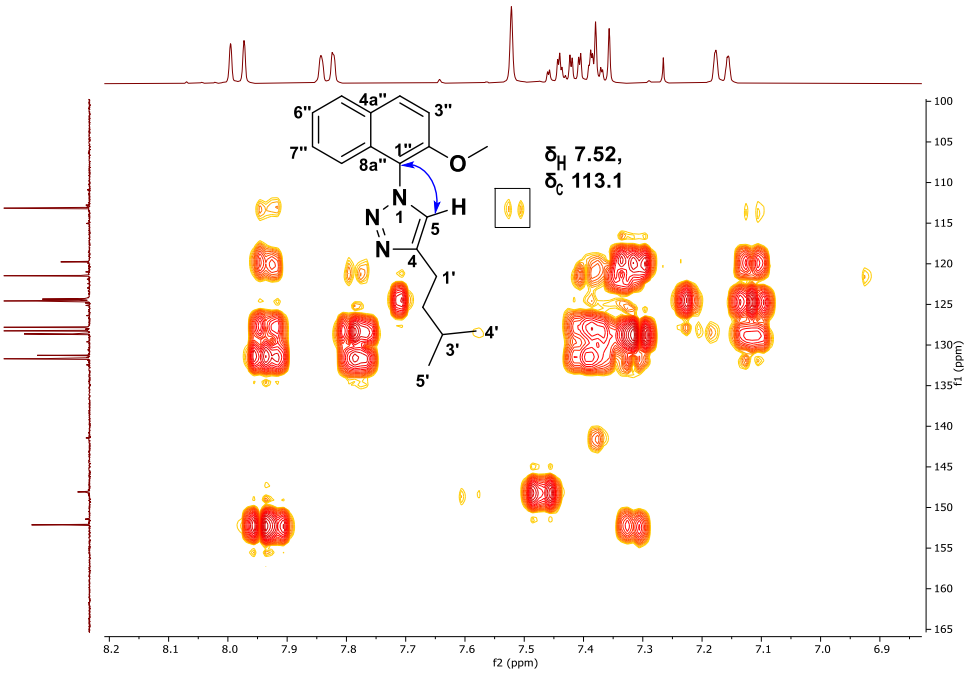


Figure 4.13: gHMBC spectrum of compound **123** (500 MHz, $CDCl_3$). The two-bond correlation between the triazole proton and C-1" is highlighted

The molecular structure of compound **123** was further confirmed by the presence of the ion peak at *m/z* 296.1771 in the HRMS (ESI) that was assigned to the protonated molecular ion ($[M + H]^+$) (calculated for $C_{18}H_{22}N_3O$ 296.1763). The CuAAC reactions of phenylazide **118** were also efficient giving the 1,4-triazoles **130 – 132** in yields of 60 – 96%. The results are shown in Scheme – 4.6. The mechanism of CuAAC reaction was discussed in section – 2.1.1.1; Scheme – 2.2 of Chapter-2.

The ^1H NMR spectrum of the 1,5-triazole **83** (Scheme – 4.2) showed the characteristic singlet resonance at δ_H 7.71 for the triazole ring proton, whereas the triazole proton in its regioisomer 1,4-triazole **123** (Scheme – 4.6) resonated at δ_H 7.52. The ^{13}C NMR spectrum of the 1,5-triazole **83** (Scheme – 4.2) showed the C-4 resonances at δ_C 131.6 and the quaternary C-5 at δ_C 140.7, whereas its regioisomeric 1,4-triazole **123** (Scheme – 4.6) showed resonances for the quaternary C-4 at δ_C 148.1 and C-5 at δ_C 124.3.

Similarly, the ^{1}H NMR spectrum of the 1,5-triazole **119** (Scheme – 4.5) revealed a characteristic singlet resonance at δ_H 7.55 for the triazole ring proton, whereas its regioisomer 1,4-triazole **130** (Scheme – 4.6) showed the corresponding resonance at δ_H 7.81. For the 1,5-triazole **119** (Scheme – 4.5), the resonance for the C-4 was at δ_C 131.3 and for the quaternary C-5 at δ_C 140.0, whereas for its regioisomer 1,4-triazole **130** (Scheme – 4.6) the C-5 and quaternary C-4 resonances were at δ_C 122.7 and δ_C 147.9, respectively.

As a general trend, the C-5 carbon resonances of the 1,4-disubstituted 1,2,3-triazoles resonated around δ_c 124 ± 3 ppm, whereas the corresponding C-4 carbon resonances of the 1,5-disubstituted 1,2,3-triazoles appeared around 132 ± 2 ppm (Figure 4.14).

Figure 4.14: ^{13}C NMR as a tool for distinguishing between 1,4- and 1,5- disubstituted 1,2,3-triazoles

In conclusion, the Mg-promoted cycloaddition reactions produced the 1,5-triazoles **83**, **100** – **105** (Scheme 4.2) in yields ranging from 52 – 89% for the azide **82** and the corresponding alkynes (i.e. 5-methyl-1-hexyne, cyclohexylacetylene, 2- and 4-methoxyphenylacetylene, 2-methylphenylacetylene, 3-fluorophenylacetylene and 2,4,6-trimethylphenylacetylene). The synthesis of the 1,5-triazoles **106** – **110** (Scheme 4.2) from azide **82** were unsuccessful due to the steric hindrance of the corresponding alkynes (2,6-dimethoxyphenylacetylene and 1-naphthylacetylene) and the electron-withdrawing effects of the corresponding alkynes (4-fluorophenylacetylene, 3- and 4-nitrophenylacetylene).

Under similar reaction conditions to those used for **82**, the reactions of the azide **97** (Scheme 4.3) with the corresponding alkynes (5-methyl-1-hexyne, 2-methoxyphenyl acetylene and 1-naphthylacetylene) were unsuccessful due to the more sterically hindered

nature of the azide **97** (2-Me substituent is more sterically demanding than the 2-OMe group of azide **82**).

Under similar reaction conditions to that for **82**, the less hindered naphthyl azide **99** gave the 1,5-triazoles **114** – **116** in yields ranging from 10 – 88% using the alkynes 5-methyl-1-hexyne, cyclohexylacetylene and 2-methoxyphenylacetylene. The reaction of the azide **99** with the more hindered 2-methoxyphenylacetylene produced the desired 1,5-triazole **116** in a low yield of 10% after heating for an extended time (12 h) (Scheme 4.4). The synthesis of the triazole **117** was unsuccessful due to the more sterically hindered nature of the alkyne (1-naphthylacetylene).

The Ru-catalysed cycloaddition reaction produced 1,5-phenyl triazoles **119 – 122** in yields ranging 7 – 87% from the reaction of the azide **118** with four different alkynes (5-methyl-1-hexyne, cyclohexylacetylene, 2-methoxyphenylacetylene and 1-naphthyl acetylene) at 70 °C for 12 – 48 h (Scheme 4.5). 1-Naphthylacetylene produced a low yield of 7% of the corresponding 1,5-triazole **122** after heating for 48 h due to its sterically hindered nature.

The Cu-catalysed cycloaddition reaction produced 1,4-triazoles **123** – **132** in yields ranging 60 – 96% from azides **82** and **118** with a variety of sterically hindered alkynes (Scheme 4.6). All of the reactions were successful and the effect of steric hindrance was observed in the case of three alkynes (5-methyl-1-hexyne, cyclohexylacetylene and 2-methoxyphenyl acetylene) as they required longer reaction times of 36 h – 48 h.

The advantages of the Grignard method over the Ru-catalyzed method is the low-cost of the reagent and its small in size allowing reactions of more hindered substrates to proceed in shorter periods of time.

The disadvantages of Ru-method – $Cp^*RuCl(PPh_3)_2$ catalyst is highly expensive, larger in size and failed to produce sterically hindered 1,5-triazoles from sterically hindered aryl azide even in a longer period of reaction time.

The magnesium-promoted ‘click’ reaction may have further applications to sterically hindered situations including, drug discovery allowing the attachment of sterically hindered chromophores for drug monitoring in biological systems, the synthesis of sterically demanding and novel chiral ligands and organocatalysts for asymmetric chemical synthesis and asymmetric chemical methodologies.

5.0 – Conclusions and future directions

A diverse set of twenty-nine novel antibacterial phenyltriazole and naphthyltriazole peptidomimetic derivatives were designed and synthesized as part of an ongoing project into the development of new antibacterial agents against *C. difficile*. This range of compounds were realized through derivatization of eleven key intermediates **58 – 68** *via* the incorporation of benzyl and alkyl substituents on the 1,2,3-triazole ring at the hydrophobic region. The eleven key intermediates **58 – 68** were achieved through implementation of a modular multi-step synthesis that utilized eight key building block precursors **50 – 57**. This modular approach should allow for easy diversification and manipulation of future synthesized derivatives based upon the resultant SAR data.

The targeted compounds were designed to incorporate an alkyl substituted naphthyl-1,2,3-triazole or a phenyl-1,2,3-triazole moiety into the hydrophobic backbone to replace the binaphthyl and biphenyl core of the lead compounds **2** and **3** (Figure 5.1); this was to improve the solubility of the peptoids, so they could be used as an oral CDI chemotherapeutic and still retain antibacterial activity. Following synthesis and spectroscopic characterization, the compounds were subjected to antimicrobial and toxicity assays to screen for improved activity. The results of these assays were used to explore the SAR trends of the compounds and thus, to identify a lead compound or other vital structural components necessary for both *C. difficile* antibacterial activity as well as general antibacterial activity.

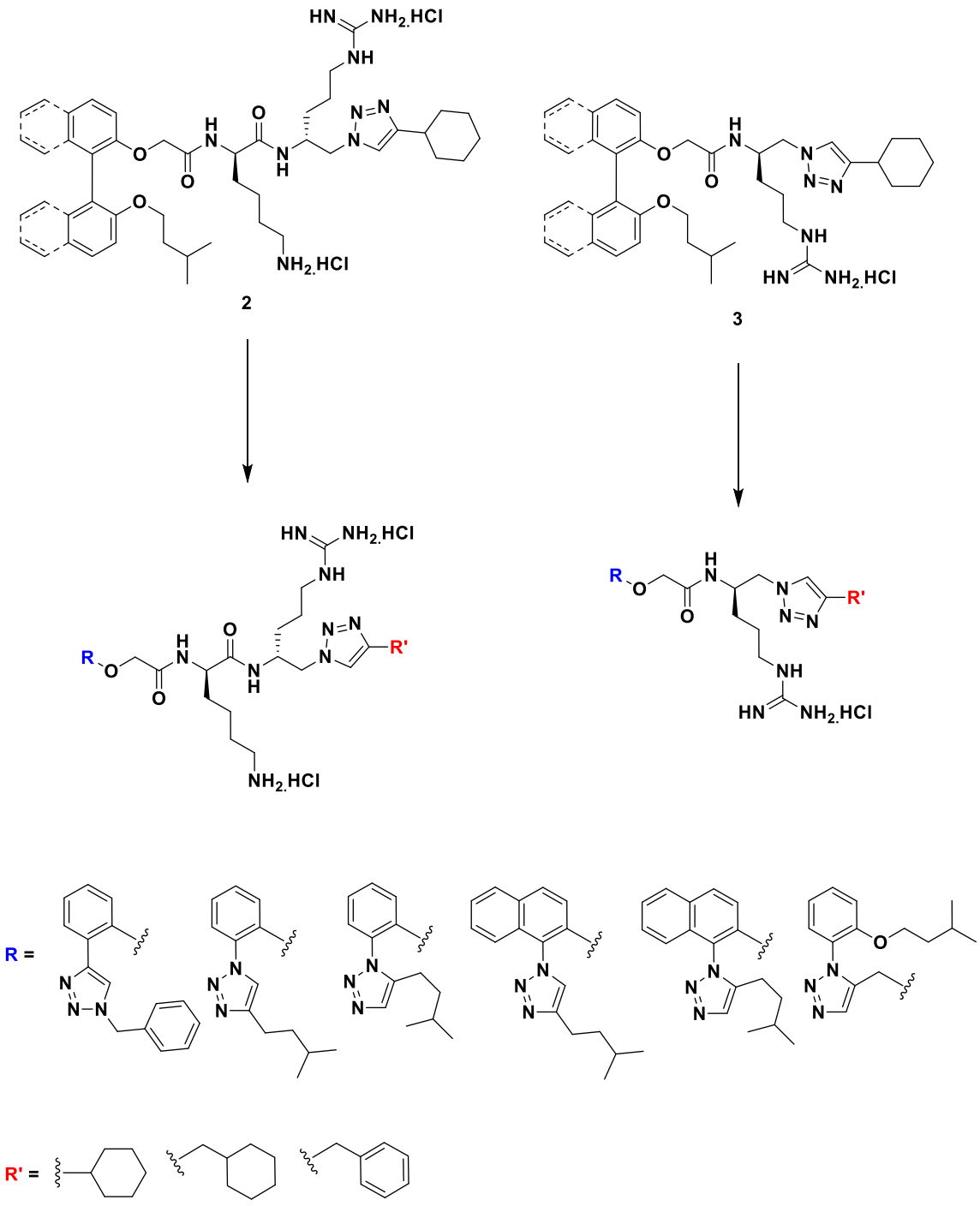


Figure 5.1 – The replacement of the biphenyl and binaphthyl core of the lead compounds **2** and **3** with alkyl substituted phenyl and naphthyl-1,2,3-triazole moieties at the hydrophobic backbone.

All synthesized compounds exhibited some level of antibacterial activity; the least potent MIC value observed against Gram-positive bacteria was 128 μg/mL, with most of the compounds having MIC values ranging from 4 – 16 μg/mL against Gram-positive bacteria.

All of the monocationic derivatives failed to exhibit antibacterial activity against *C. difficile*. The dicationic derivatives (**41** and **43**) exhibited less Gram-positive antibacterial efficacy but exhibited antibacterial activity against the *C. difficile* strains with MIC values of 8 μg/mL (Figure 5.2).

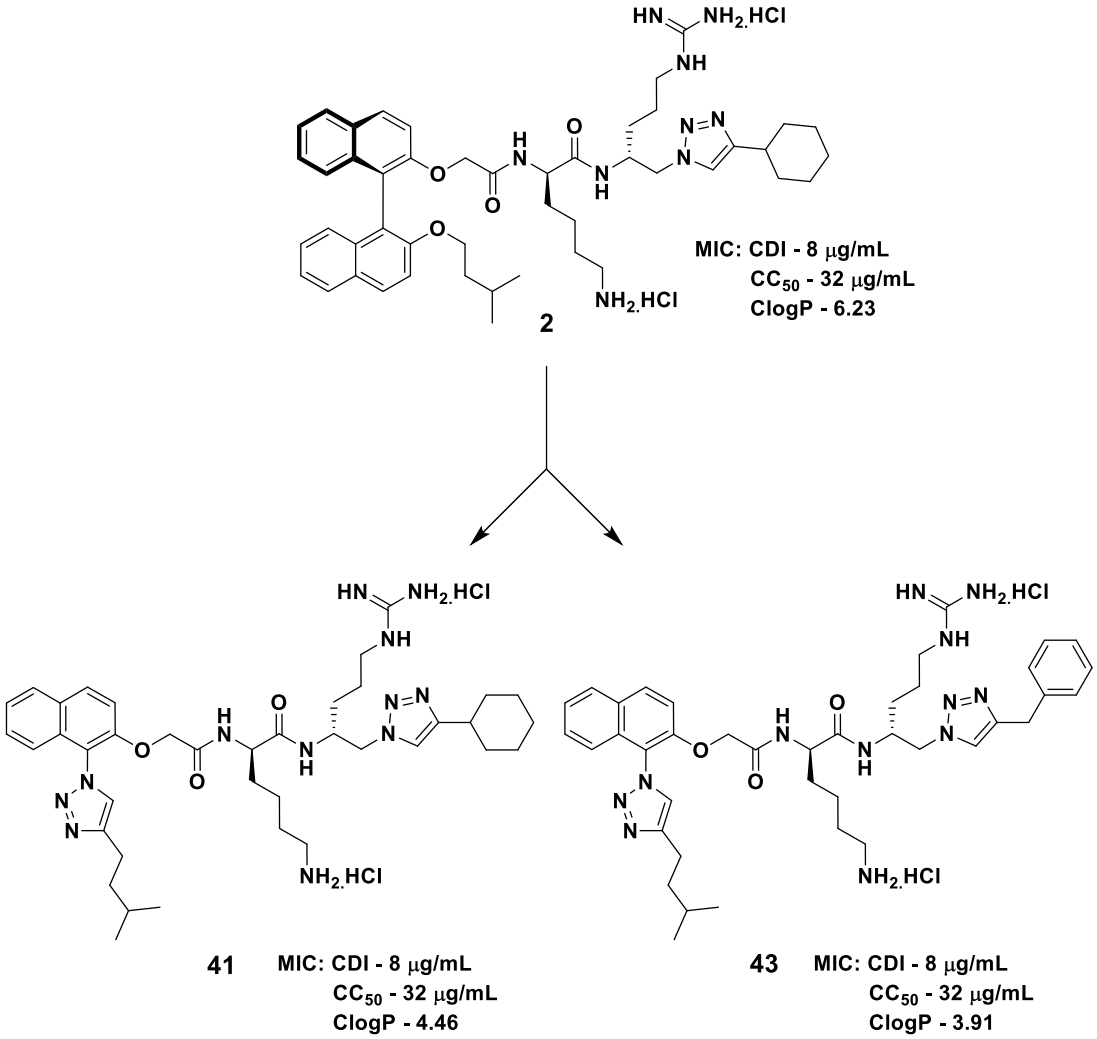


Figure 5.2 – Development of the potential CDI hit compounds **41** and **43** from lead compound **2**.

These dicationic derivatives **41** and **42** exhibited fungal activity against *C. neoformans*. Compound **2** exhibited the strongest broad-spectrum antibacterial efficacy, but it had poor water solubility. Compounds **41** and **43** exhibit less broad-spectrum antibacterial efficacy than compound **2** but they had five times better water solubility. The replacement of the binaphthyl group with a phenyl-1,2,3-triazole or naphthyl-1,2,3-triazole moiety led to increased compound solubility which often translated to a decrease in Gram-positive antibacterial activity.

As seen in Figure 5.2, the novel antibacterial molecules **41** and **43**, designed from compound **2** led to the development of distinct structural derivatives that exhibited an increase in desirable properties for an antibacterial chemotherapeutic. Furthermore, compound **41** exhibited antibacterial activity against *C. difficile*.

Compound **41** was selected as a potential hit compound based upon its *in vitro* activity against *C. difficile* and good water solubility. It was synthesized on larger scale and used in an *in vivo* CDI mouse model at Monash University. The data of the CDI mouse model trial revealed that mice fed compound **41** showed 80% survival rate after 24 h; both mouse weight loss and disease progression were significantly slowed by compound **41** during the administration of the trial. Compound **41** exhibited selectivity for Gram-positive bacteria – it was much less active against all the Gram-negative bacteria that were tested. This selectivity could be the reason for the drugs positive performance in the CDI mouse model study; by not eliminating the commensal, Gram-negative *Bacteroides* spp. and *E. coli*, compound **41** was likely quite effective at selectively killing *C. difficile* without destroying the entire enteric microflora. If successful in further antibacterial trials, the compound **41** will be studied and possibly developed as a potential CDI chemotherapeutic.

A solubility assay was also developed and performed on selected compounds to ascertain the role of solubility in the efficacy and administration of the phenyl and naphthyl peptide derivatives.

In summary, a total of 29 novel antibacterial compounds were synthesized and fully characterized. The synthesis of these derivatives involved the synthesis of 96 intermediate compounds in total, i.e., 87 novel intermediate compounds and nine known compounds. A modular synthetic route to various novel phenyltriazole and naphthyltriazole peptide scaffolds was realized and the resultant synthetic derivatives represent a potential new direction for developing novel antibiotics for the treatment of CDI and other bacterial infections. The compounds synthesized and identified in this project are the subject of ongoing studies into the development of a novel CDI treatment. Therefore, the current and future chemical and biological research focused on these phenyl and naphthyl peptide derivatives will aim towards the advancement of an effective and potent CDI chemotherapeutic.

Future drug discovery research will likely involve two target areas. One area will focus on scale-up of compound **43** for further *in vivo* mouse model study and the second target area will likely focus on modifications of compounds **44 – 46**. These modifications might produce novel peptidomimetics with good water solubility and increasing antibacterial efficacy – this is an important biological study requirement needed for novel medications that work against drug-resistant *C. difficile* and other Gram-positive bacteria.

During the synthetic part of this project, it was revealed that the synthesis of sterically hindered 1,5-disubstituted-1,2,3-triazoles from hindered aryl azides and alkynes was not successful using $Cp^*RuCl(PPh_3)_2$ as a catalyst due to the sterically hindered nature of the catalyst and the azide substrate. Also, PPh_3 derived from the $Cp^*RuCl(PPh_3)_2$ catalyst

converted some of the azide to an amine in an undesired reaction. This led to a study of the magnesium-promoted click reaction which was initially developed by Sharpless. *et al*.[92] The study involved the regioselective synthesis of 1,5-disubstituted-1,2,3-triazole *via* the addition of chloromagnesium acetylides to azides. By applying this methodology, sterically hindered 1,5-disubstituted-1,2,3-triazoles were prepared by treating chloromagnesium acetylides with less and more hindered azides in yields ranging from 52-89%.

In summary, the lead compound **1** (**AVX-13616**; Figure 1.11) and its derivatives **2** and **3** displayed exceptional *in vitro* antimicrobial potency (MIC) of 8.0 µg/mL against hypervirulent *C. difficile* (Figure 1.12 and 1.13)[54]. These compounds had poor water solubility making mouse models studies difficult and the results unreliable. In this study we have designed and synthesized new compounds having related cationic peptidomimetic features to compounds **2** and **3** with modifications of the hydrophobic 1,1'-binaphthyl moiety to *N*-phenyltriazole and *N*-naphthyltriazole moieties. These modifications produced better water-soluble compounds and for compound **41** and **43** retained antimicrobial potency (MIC) of 8.0 µg/mL against *C. difficile*. The data of the CDI mouse model trial revealed that mice fed compound **41** showed 80% survival rate after 24 h; both mouse weight loss and disease progression were significantly slowed by compound **41** during the administration of the trial. Based on analysis of these results, future research will be focused on the scale up of compound **43** for *in vivo* mouse model studies.

6.0 – Experimental

6.1 – General information

Synthesis

Unless otherwise stated, all solvents and chemicals were laboratory or reagent grade and were purchased from commercial sources and used as received. Water was purified *via* Millipore filtration prior to use. HOBt was purchased with added stabilizers (10% w/w H_2O); therefore, the quantities required for reactions were adjusted accordingly and are reflected in the reagent masses reported in the experimental (whereas the reported mmol quantities reflect the true quantity of a chemical). All reactions were conducted under normal atmosphere and cold reaction temperatures were obtained by an ice bath (0 ºC) or ice/salt bath (–10 ºC). Heating of reactions was performed with a paraffin oil bath. Small quantities of liquid reagents were measured and added to reactions *via* syringe or autopipette. Unless otherwise noted, all filtrations were conducted as vacuum filtration through a sintered glass funnel (medium porosity). Vacuum filtration was achieved with the aid of a water aspirator. Solvent removal *via* concentration was performed on a rotary evaporator under reduced pressure. All solvent mixtures are expressed in terms of volume ratio (i.e. v/v). Thin layer chromatography (TLC) was performed on aluminium-backed SiO_2 gel plates (F254 grade - 0.20 mm thickness). Visualization was achieved with UV light, ninhydrin stain or cerium ammonium molybdate stain. Flash chromatography was performed on SiO_2 gel 60 with a positive air pressure. All synthesized compounds were dried under high vacuum (< 1 mbar) before determination of chemical yields and spectroscopic characterization. Optical rotations of only the final deprotected compounds were recorded rather than non-final compounds.

Characterization and analysis

^{1}H NMR spectra were recorded on a Bruker Avance 400 (400 MHz), Avance 500 (500 MHz), a Varian VNMRS PS54 500 (500 MHz), a Varian Inova 500 (500 MHz) or a Varian Mercury 400 (400 MHz) NMR spectrometer. Chemical shifts are reported in ppm and were measured relative to the internal standard. Samples were dissolved in $CDCl_3$ (with TMS as the internal standard – 0.00 ppm), CD_3OD (solvent resonance as internal standard – 3.31 ppm) or DMSO-*d*6 (solvent resonance as internal standard – 2.50 ppm). The ^{1}H NMR data is reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, dt = doublet of triplets, m = multiplet, br = broad), coupling constants (Hz), integration and assignment ^{13}C NMR spectra were recorded on a Bruker Avance 400 (101 MHz), Avance 500 (126 MHz), a Varian VNMRS PS54 500 (126 MHz), a Varian Inova 500 (126 MHz) or a Varian Mercury 400 (101 MHz) NMR spectrometer with complete ^{1}H decoupling. Chemical shifts are reported in ppm and were measured relative to the internal standard. Samples were dissolved in $CDCl_3$ (solvent resonance as the internal standard – 77.16 ppm), CD_3OD (solvent resonance as the internal standard – 49.20 ppm) or DMSO-d_6 (solvent resonance as internal standard – 39.50 ppm). Variable temperature NMR experiments were performed at 90 °C on the Bruker Avance 400 NMR spectrometer. ^{1}H and ^{13}C NMR resonances assignments were confirmed by analysis of NMR experiments: APT, gCOSY, gHSQC, gHMBC, zTOCSY and NOESY. Carbon resonances that required 2-D NMR analysis for assignment (i.e. not observed *via* 1-D ^{13}C NMR analysis) are marked with the label "*observed by gHMBC*" or "*observed by gHSQC*". Compounds that exhibited non-observable carbon resonances in both 1-D and 2-D NMR analysis are denoted and explained with footnotes. Unassigned aromatic hydrogens are labelled as "Ar", whereas specific/assigned aromatic hydrogens are labelled with the normal nomenclature (i.e. Ar3 =

hydrogen atom attached to aromatic carbon #3). NMR spectra were processed and prepared with MestReNova (version 6.0) NMR software. Low resolution mass spectra (LRMS) were obtained *via* electrospray ionization (ESI) on a Shimadzu LC-2010 mass spectrometer. LRMS data was recorded as the ion mass/charge ratio (*m*/*z*). High resolution mass spectrometry (HRMS) was performed on a Waters Quadrupole-Time of Flight (QTOF) Xevo spectrometer *via* ESI and with Leucine-Enkephalin as an internal standard. All mass spectrometry samples were dissolved in high performance liquid chromatography (HPLC) grade MeOH (containing <1% formic acid for ionization). Optical rotations were measured on a Jasco P-2000 polarimeter with a 10 cm path length; rotation values (α) are expressed in units of "deg cm^3 g^{-1} dm^{-1}" with concentration (*c*) expressed in units of "g/100 mL". Solid-state infrared spectroscopy was performed on a Shimadzu IRAffinity-1 FTIR spectrometer in combination with a MIRacle 10 Single Reflection Attenuated Total Reflectance accessory outfitted with a 1.5 mm round diamond crystal. IR peaks are reported as the wavenumber ($\bar{\nu}_{max}$ in cm^{-1}) of the maximum absorption.

6.2 – General synthetic procedures

General Procedure I: Alkylation of phenols (with ethyl bromoacetate)

To a stirred solution of the phenol (1 eq) in dry DMF (5 mL/mmol substrate) was added K_2CO_3 (3 eq), followed by ethyl bromoacetate (1.3 eq) at room temperature and the reaction was allowed to stir at rt for 12 h. The reaction mixture was diluted with EtOAc (2 x 50 mL), washed with water (2 x 50 mL), brine (2 x 50 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel to afford the desired ester product.

General Procedure II: Ester hydrolysis (with KOH)

To a stirred solution of the ester (1 eq) in ethanol (10 mL/mmol substrate) was added 7% KOH solution (5 mL/mmol) at rt and the reaction was allowed to stir at rt for 2 h. The reaction mixture was acidified with 1 M HCl (25 mL) and extracted with EtOAc (2 x 25 mL). The combined organic extracts were washed with brine (50 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum to afford the acid product.

General Procedure III: Amide coupling

The amine (1.0 eq), carboxylic acid (1.0 eq), EDC.HCl (1.2 eq), HOBt (1.1 eq) and TEA (1 eq) were combined in a dichloromethane/acetonitrile solution (10 mL/mmol amine) and stirred at rt for the specified time. The solvent was removed (not required for ≤ 5.0 mL dichloromethane/acetonitrile) and the residue was dissolved in EtOAc (25 mL for reactions that contained ≤ 1.0 mmol amine or 25 mL/mmol amine for larger scale reactions). The organic solution was washed successively with aqueous HCl (1.0 M – 2 x 25 mL), saturated aqueous $NaHCO_3$ (3 x 25 mL) and brine (1 x 25 mL). The EtOAc solution was dried ($MgSO_4$), filtered and concentrated. If necessary, the residue was subjected to further purification *via* flash chromatography over SiO_2 gel to furnish the targeted amide product.

General Procedure IV: Copper-catalyzed azide-alkyne cycloaddition

To a stirred solution of the azide (1.0 eq) and alkyne (2.0 – 3.0 eq) in *tert*-butanol/water (4:1) at rt was added $CuSO_4 \cdot 5H_2O$ (0.2 eq), followed by sodium ascorbate (0.4 eq). Stirring was continued at rt (unless noted otherwise) for the specified time. Aqueous saturated NH_4Cl solution (1 mL), then water (20 mL) was added, and the mixture was extracted with EtOAc (20 mL for reactions that contained ≤ 1.0 mmol azide or 20 mL/mmol azide for larger scale reactions). The combined extracts were washed with water (2 x 25 mL),

brine (2 x 25 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to flash chromatography over SiO_2 gel to afford the desired 1,4-disubstituted 1,2,3-triazole product.

General Procedure V: Ruthenium-catalyzed azide-alkyne cycloaddition

To a stirred solution of the azide (1 eq) in dry 1,4-dioxane (2 mL/mmol substrate), in a dried flask equipped with a condenser and under an atmosphere of nitrogen at room temperature, was added the alkyne (1.1 eq), followed by pentamethylcyclopentadienylbis (triphenylphosphine)ruthenium(II) chloride (0.1 eq). The mixture was stirred and heated at 70 °C for 12 h. The reaction mixture was cooled to room temperature and saturated aqueous NH_4Cl solution (1 mL), then water (25 mL) was added, and the mixture was extracted with EtOAc (2 x 25 mL). The combined extracts were washed with water (2 x 25 mL), brine (2 x 25 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to flash chromatography over SiO_2 gel to afford the desired 1,5-disubstituted 1,2,3-triazole product.

General Procedure VI: Magnesium-promoted azide-alkyne cycloaddition

To a dried flask equipped with a condenser and containing a solution of EtMgCl (2 M in diethyl ether, 1.2 eq) in dry THF (10 mL/mmol) under a nitrogen atmosphere at room temperature, was added dropwise, *via* a syringe, the alkyne (1.1 eq). The mixture was stirred and heated at 50 °C for 30 min. The solution was cooled to rt and a solution of the azide (1 eq) in dry THF (10 mL/mmol) was added dropwise over a period of 5 min at rt. After a further 10 min, the reaction mixture was stirred and heated at 50 °C for 3-5 h. Aqueous saturated NH_4Cl solution (1 mL), then water (25 mL) was added, and the mixture was extracted with

EtOAc (2 x 25 mL). The combined extracts were washed with water (2 x 25 mL), brine (2 x 25 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel to afford the desired 1,5-disubstituted 1,2,3-triazole product.

General Procedure VII: Amine deprotection (*N*-Boc and/or *N*-Pbf removal)

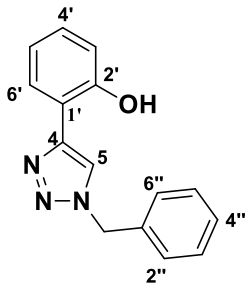
The *N*-protected amine (1.0 eq) was dissolved in a CH_2Cl_2 (30 mL/mmol substrate) with magnetic stirring. If the substrate molecule contained an *N*-Pbf moiety then H_2O (20.0 eq) was also added to the solution. TFA (30.0 mL/mmol substrate) was then added and the reaction mixture was stirred at rt overnight (> 16 h) followed by removal of the solvent. The residue was dissolved in CH_2Cl_2 (30 mL/mmol substrate), an excess amount of anhydrous HCl (2.0 M in Et_2O, 15 mL/mmol substrate, 30.0 eq) was added and the solvent was then removed. The resulting residue was dissolved in a minimal volume of CH_2Cl_2 (or MeOH) and excess Et_2O (25 mL for $\leq$ 0.1 mmol substrate) was added to precipitate the hydrochloride salt of the amine. The reaction mixture was filtered, resulting filtrate was collected and concentrated, then triturated with Et_2O (3 x 20 mL). The product was collected by dissolution in MeOH; concentration followed by drying *in vacuo* gave the final mono or di-hydrochloride salt as a thin, translucent film that was routinely scratched with a spatula into a fine hygroscopic powder or amorphous gum.

6.3 – Synthesis

6.3.1 – Precursor Building Blocks (Aromatic Cores)

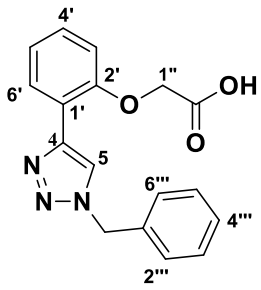
6.3.1.1 – Triazole linked phenolic acids

2-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)phenol (70)



Following **General Procedure IV**, benzyl azide (1.00 g, 7.51 mmol, 0.5 M in CH_2Cl_2), 2-ethynylphenol **69** (0.89 g, 7.51 mmol), $CuSO_4{\cdot}5H_2O$ (0.37 g, 1.50 mmol) and sodium ascorbate (0.59 g, 3.01 mmol) were stirred in *t*-BuOH (10 mL) and H_2O (2.5 mL) at rt for 12 h to give the triazole **70** (1.51 g, 80%) as a translucent tan gum after flash chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80). TLC (EtOAc/*n*-hexane - 40:60): R_f = 0.6; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.82 (brs, 1H, -OH), 7.72 (s, 1H, H5), 7.41-7.39 (m, 3H, H6', H3"/H5"), 7.34-7.30 (m, 3H, H2"/H4"/H6"), 7.23 (apparent t, *J* = 8.0 Hz, 1H, H4'), 7.04 (d, *J* = 8.0 Hz, 1H, H3'), 6.85 (apparent t, *J* = 8.0 Hz, 1H, H5'), 5.59 (s, 2H, C$\underline{H}_2$Ph); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.0 (C2'), 148.3 (C4), 134.2 (C1"), 129.9 (C4'), 129.4 (C3"/C5"), 129.2 (C2"/C6"), 128.3 (C6'), 125.9 (C5), 119.5 (C4"), 118.8 (C5'), 117.8 (C1'), 114.0 (C3'), 54.7 ($\underline{C}H_2$Bn); IR (neat) $\bar{\nu}_{max}$ 3021, 3138, 3033, 1617, 1580, 1478, 1449, 1356, 1277, 1247, 1235, 1213, 1157, 1071, 1056, 1033, 728, 692, 653 cm^{-1}; MS (ESI +ve) *m/z* 252 ($[M + H]^+$, 100%) ; HRMS (ESI +ve TOF) calcd for $C_{15}H_{14}N_3O$ 252.1137, found 252.1136 ($[M+H]^+$).

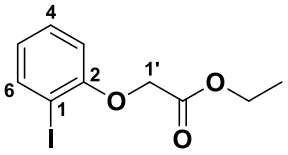
2-(2-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)phenoxy)acetic acid (50)



To a stirred solution of 2-(1-benzyl-1*H*-1,2,3-triazol-4-yl)phenol **70** (1.50 g, 5.97 mmol) in dry THF (10 mL) was added K_2CO_3 (2.47 g, 17.92 mmol), followed by bromoacetic acid (1.24 g, 8.95 mmol) at rt and the reaction was stirred and heated at 80 °C for 12 h. The reaction mixture was cooled to rt, diluted with water (50 mL) and extracted with Et_2O (50 mL). The aqueous layer was cooled to 0 °C, acidified with 1 M HCl solution (25 mL) and the resultant white color precipitate was filtered and dried under vacuum to afford the acid **50** (1.56 g,

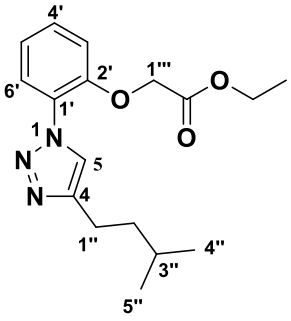
80%) as a white solid. M.P: 138 - 140 °C. TLC (MeOH/CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H, H5), 8.23 (d, *J* = 8.0 Hz, 1H, H6'), 7.40-7.33 (m, 5H, H2'''/H3'''/H4'''/H5'''/H6'''), 7.30 (apparent t, *J* = 8.3 Hz, 1H, H4'), 7.11 (apparent t, *J* = 8.0 Hz, 1H, H5'), 6.88 (d, *J* = 8.3 Hz, 1H, H3'), 5.60 (s, 2H, C$\underline{H}_2$Ph), 4.68 (s, 2H, H1''), COOH resonance was not observed; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (C=O), 153.7 (C2'), 143.3 (C4), 134.9 (C1'''), 129.0 (C3'''/C5'''), 128.9 (C4'), 128.5 (C2'''/C6'''), 127.9 (C6'), 127.4 (C5), 124.3 (C4'''), 121.9 (C5'), 119.5 (C1'), 111.8 (C3'), 65.3 (C1''), 54.1 (C$\underline{H}_2$Ph); IR (neat) $\bar{\nu}_{max}$ 2950, 1735, 1608, 1586, 1550, 1490, 1438, 1351, 1290, 1224, 1126, 1078, 1047, 835, 756, 725, 696, 648 cm^{-1}; MS (ESI +ve) *m/z* 348 ($[M + K]^+$, 100%), 310 ($[M + H]^+$, 25%); HRMS (ESI +ve TOF) calcd for $C_{17}H_{15}N_3O_3Na$ 332.1011, found 332.1003 ($[M + Na]^+$).

Ethyl 2-(2-iodophenoxy)acetate (72)[78]



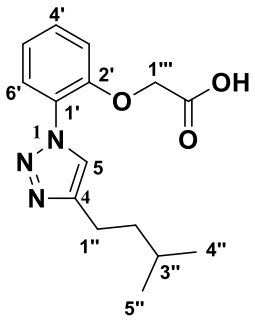
Following **General Procedure I**, 1-iodophenol **71** (5.00 g, 22.72 mmol), K_2CO_3 (9.42 g, 68.18 mmol) and ethyl bromoacetate (4.93 g, 29.54 mmol) were stirred in dry DMF (40 mL) at rt for 16 h to give the ester **72** (6.52 g, 93%) as a pale brown oil after flash chromatography over SiO_2 gel (EtOAc/*n*-hexane - 10:90). The spectroscopic data was found to be in agreement with those previously reported.[78] TLC (EtOAc/*n*-hexane - 20:80): R_f = 0.6; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 8.0 Hz, 1H, H6), 7.28-7.25 (m, 1H, H4), 6.78-6.71 (m, 2H, H3/H5), 4.67 (s, 2H, H1'), 4.25 (q, *J* = 7.0 Hz, 2H, OC$\underline{H}_2$CH$_3$), 1.28 (t, *J* = 7.0 Hz, 3H, OCH$_2$C$\underline{H}_3$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.3 (C=O), 156.8 (C2), 139.7 (C6), 129.6 (C4), 123.6 (C5), 112.6 (C3), 86.5 (C1), 66.5 (C1') , 61.6 (O$\underline{C}$H$_2$CH$_3$), 14.1 (OCH$_2\underline{C}$H$_3$); IR (neat) $\bar{\nu}_{max}$ 3480, 2984, 2940, 1743, 1621, 1501, 1461, 1448, 1379, 1350, 1293, 1209, 1152, 1104, 802, 763 cm^{-1}; MS (ESI +ve) *m/z* 329 ($[M + Na]^+$, 100%).

Ethyl 2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetate (73)



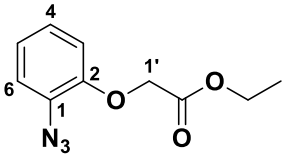
To a stirred solution of ethyl 2-(2-iodophenoxy)acetate **72** (1.00 g, 3.26 mmol), 5-methyl-1-hexyne (0.94 g, 9.80 mmol), CuI (0.12 g, 0.65 mmol), NaN_3 (0.23 g, 3.59 mmol) and sodium ascorbate (0.26 g, 1.30 mmol) in DMSO (10 mL)/H_2O (2 mL) was added racemic *trans*-*N*,*N'*-dimethylcyclohexane-1,2-diamine (0.09 g, 0.65 mmol) at rt under nitrogen atmosphere. The reaction mixture was stirred and heated at 75 °C for 16 h. The reaction was cooled to rt and aqueous saturated NH_4Cl solution (10 mL) was added and the mixture was extracted with EtOAc (2 x 50 mL). The combined extracts were washed with water (50 mL), brine (50 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 10:90 → 100:0) to afford **73** (0.91 g, 87%) as a yellow waxy solid. TLC (EtOAc/*n*-hexane - 20:80); R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.16 (s, 1H, H5), 7.87 (d, *J* = 7.5 Hz, 1H, H6'), 7.35 (apparent t, *J* = 7.5 Hz, 1H, H4'), 7.13 (apparent t, *J* = 7.5 Hz, 1H, H5'), 6.96 (d, *J* = 7.5 Hz, 1H, H3'), 4.68 (s, 2H, H1'''), 4.25 (q, *J* = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 2.81 (t, *J* = 7.5 Hz, 2H, H1''), 1.70-1.61 (m, 3H, H2''/H3''), 1.29 (t, *J* = 7.5 Hz, 3H, $OCH_2C\underline{H}_3$), 0.96 (d, *J* = 6.0 Hz, 6H, H4''/H5''); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.0 (C=O), 149.0 (C2'), 148.1 (C4), 129.4 (C4'), 127.2 (C6'), 125.4 (C5'), 123.1 (C5), 122.4 (C3'), 113.3 (C1'), 65.9 (C1'''), 61.6 ($O\underline{C}H_2CH_3$), 38.5 (C2''), 27.7 (C1''), 23.7 (C3''), 22.4 (C4''/C5''; Observed by gHMBC), 14.1 ($OCH_2\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 2957, 2870, 1755, 1601, 1548, 1509, 1466, 1379, 1296, 1230, 1203, 1167, 1129, 1073, 977, 758 cm^{-1}; MS (ESI +ve) *m/z* 318 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{17}H_{24}N_3O_3$ 318.1818, found 318.1831 ([M + H]$^+$).

2-(2-(4-Isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetic acid (51)



Following **General Procedure II**, ethyl 2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy) acetate **73** (0.90 g, 2.84 mmol) and 7% KOH solution (3 mL) were stirred in ethanol (10 mL) at rt for 2 h to give the product acid **51** (0.71 g, 85%) as a white solid. M.P: 118 - 120 °C. TLC (EtOAc/*n*-hexane - 100:0): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.36 (brs, 1H, COOH), 8.19 (s, 1H, H5), 7.77 (d, *J* = 7.5 Hz, 1H, H6'), 7.39 (apparent t, *J* = 7.5 Hz, 1H, H4'), 7.13 (apparent t, *J* = 7.5 Hz, 1H, H5'), 7.04 (d, *J* = 7.5 Hz, 1H, H3'), 4.76 (s, 2H, H1'''), 2.80 (t, *J* = 7.5 Hz, 2H, H1''), 1.64-1.59 (m, 3H, H2''/H3''), 0.92 (d, *J* = 5.5 Hz, 6H, H4''/H5''); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.8 (C=O), 149.4 (C2'), 147.9 (C4), 130.2 (C4'), 126.7 (C6'), 125.4 (C5'), 123.7 (C5), 122.5 (C3'), 113.6 (C1'), 65.9 (C1'''), 38.3 (C2''), 27.7 (C1''), 23.2 (C3''), 22.4 (C4''/5''; Observed by gHMBC); IR (neat) $\bar{\nu}_{max}$ 3160, 2955, 2930, 1737, 1602, 1505 1471, 1439, 1294, 1219, 1181, 1129, 1065, 835, 753 cm^{-1}; MS (ESI +ve) *m/z* 290 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{15}H_{20}N_3O_3$ 290.1505, found 290.1513 ([M + H]$^+$).

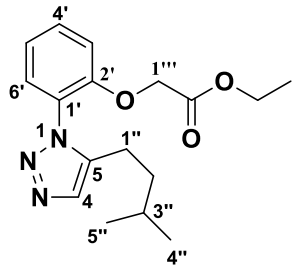
Ethyl 2-(2-azidophenoxy)acetate (77)



To a stirred solution of ethyl 2-(2-iodophenoxy)acetate **72** (1.00 g, 3.26 mmol), CuI (0.06 g, 0.32 mmol), NaN_3 (0.24 g, 3.59 mmol) and sodium ascorbate (0.06 g, 0.32 mmol) in DMSO (10 mL)/H_2O (2 mL) was added racemic *trans-N,N'*-dimethylcyclohexane-1,2-diamine (0.07 g, 0.49 mmol) at rt under nitrogen atmosphere. The reaction mixture was stirred and heated at 80 °C for 5 h. The reaction was diluted with water (100 mL) and extracted with EtOAc (2 x 100 mL). The combined extracts were washed with water (100 mL), brine (50 mL) and dried ($MgSO_4$). The

solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 10:90 → 100:0) to afford **77** (0.51 g, 70%) as a yellow waxy solid. TLC (EtOAc/CH_2Cl_2/*n*-hexane – 10:10:80); R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08-6.95 (m, 3H, H4/H5/H6), 6.81 (dd, *J* = 8.1, 1.3 Hz, 1H, H3), 4.67 (s, 2H, H1'), 4.26 (q, *J* = 7.1 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 1.28 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.3 (C=O), 150.2 (C2), 129.1 (C1), 125.4 (C6), 122.6 (C4), 120.7 (C5), 113.8 (C3), 66.2 (C1'), 61.4 (OC<u>H</u>$_2$CH$_3$), 14.12 (OCH$_2$C<u>H</u>$_3$); IR (neat) $\bar{\nu}_{max}$ 2984, 2116, 1756, 1738, 1592, 1495, 1473, 1454, 1379, 1297, 1199, 1106, 1072, 1020, 749 cm^{-1}; MS (ESI +ve) *m/z* 194 ([M + H – N_2]$^+$, 100%).

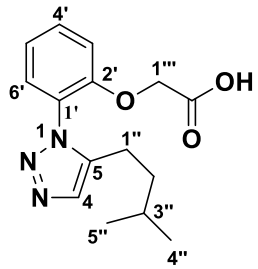
Ethyl 2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetate (78)



Following **General Procedure V**, ethyl 2-(2-azidophenoxy)acetate **77** (0.53 g, 2.40 mmol), 5-methyl-1-hexyne (0.46 g, 4.80 mmol) and pentamethylcyclopentadienylbis(triphenylphosphine) ruthenium(II) chloride (0.19 g, 0.24 mmol) were stirred and heated in 1,4-dioxane at 70 °C for 12 h to give the ester **78** (0.28 g, 37%) as a pale-yellow oil after flash chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80). TLC (EtOAc/*n*-hexane – 50:50): R_f = 0.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H, H4), 7.46 (apparent t, *J* = 6.8 Hz, 1H, H4'), 7.37 (d, *J* = 6.4 Hz, 1H, H6'), 7.14 (apparent t, *J* = 6.4 Hz, 1H, H5'), 6.92 (d, *J* = 6.8 Hz, 1H, H3'), 4.55 (s, 2H, H1'''), 4.20 (q, *J* = 5.6 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 2.64-2.56 (m, 2H, H1''), 1.56-1.42 (m, 3H, H2''/H3''), 1.24 (t, *J* = 5.6 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 0.81 (d, *J* = 5.2 Hz, 6H, H4''/H5''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.1 (C=O), 152.6 (C2'), 140.6 (C5), 131.5 (C4), 131.5 (C4'), 129.2 (C6'), 125.8 (C1'), 122.2 (C5'), 113.2 (C3'), 65.7 (C1'''), 61.6 (O<u>C</u>H$_2$CH$_3$), 37.0 (C2''), 27.6 (C1''), 22.3 (C4''/C5''), 21.2 (C3''), 14.2 (OCH$_2$<u>C</u>H$_3$); IR (neat) $\bar{\nu}_{max}$ 2957,

2935, 2870, 1755, 1602, 1509, 1466, 1445, 1381, 1369, 1295, 1203, 1167, 1129, 1073, 1025, 977, 757 cm^{-1}; MS (ESI +ve) *m/z* 340 ($[M + Na]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{17}H_{23}N_3O_3Na$ 340.1637, found 340.1638 ($[M + Na]^+$).

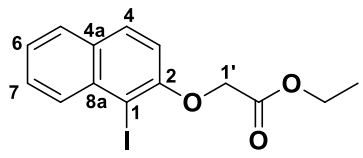
2-(2-(5-Isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetic acid (52)



Following **General Procedure II**, ethyl 2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetate **78** (0.28 g, 0.88 mmol) and 7% KOH solution (4 mL) were stirred in ethanol (4 mL) at rt for 2 h to give the product acid **52** (0.21 g, 78%) as a pale brown solid. M.P: 128-130 °C. TLC (EtOAc/*n*-hexane - 100:0): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.10 (brs, 1H, COOH), 7.62 (s, 1H, H4), 7.49 (apparent t, *J* = 8.0 Hz, 1H, H4'), 7.35 (d, *J* = 7.5 Hz, 1H, H6'), 7.15 (apparent t, *J* = 7.5 Hz, 1H, H5'), 7.03 (d, *J* = 8.0 Hz, 1H, H3'), 4.64 (s, 2H, H1'''), 2.61 (t, *J* = 7.5 Hz, 2H, H1''), 1.56-1.42 (m, 3H, H2''/H3''), 0.81 (d, *J* = 6.5 Hz, 6H, H4''/H5''); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (C=O), 152.4 (C2'), 140.6 (C5), 131.9 (C4), 131.6 (C4'), 128.5 (C6'), 125.6 (C1'), 122.6 (C5'), 114.1 (C3'), 66.0 (C1'''), 37.1 (C2''), 27.6 (C1''), 22.3 (C4''/C5''; Observed by gHMBC), 21.3 (C3''); IR (neat) $\bar{\nu}_{max}$ 2960, 2929, 2871, 1722, 1602, 1509, 1465, 1371, 1296, 1231, 1164, 1110, 1072, 995, 840, 760 cm^{-1}; MS (ESI +ve) *m/z* 328 ($[M + K]^+$, 100%), 290 ($[M + H]^+$, 60%); HRMS (ESI +ve TOF) calcd for $C_{15}H_{19}N_3O_3Na$ 312.1324, found 312.1329 ($[M + Na]^+$).

6.3.1.2 – Triazole linked naphthalic acids

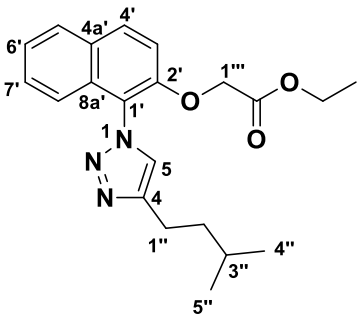
Ethyl 2-((1-iodonaphthalen-2-yl)oxy)acetate (75)



Following **General Procedure I**, 1-iodonaphthol **74** (1.00 g, 3.70 mmol), K_2CO_3 (1.53 g, 11.11 mmol) and ethyl

bromoacetate (0.80 g, 4.81 mmol) were stirred in DMF (8 mL) at rt for 16 h to give the ester **75** (0.68 g, 52%) as a pale yellow waxy solid after flash chromatography over SiO_2 gel (EtOAc/*n*-hexane - 10:90). TLC (EtOAc/*n*-hexane - 20:80): R_f = 0.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, *J* = 7.2 Hz, 1H, H8), 7.78 (d, *J* = 7.2 Hz, 1H, H5), 7.72 (d, *J* = 8.0 Hz, 1H, H4), 7.54 (t, *J* = 7.2 Hz, 1H, H7), 7.39 (t, *J* = 7.2 Hz, 1H, H6), 7.08 (d, *J* = 8.0 Hz, 1H, H3), 4.80 (s, 2H, H1'), 4.27 (q, *J* = 5.6 Hz, 2H, $OC\underline{H}_2CH_3$), 1.29 (t, *J* = 5.6 Hz, 3H, $OCH_2C\underline{H}_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.7 (C=O), 155.6 (C2), 135.8 (C8a), 131.7 (C4a), 130.6 (C4), 130.5 (C8), 128.4 (C7), 128.3 (C5), 121.1 (C6), 114.4 (C3), 89.47 (C1), 67.6 (C1'), 61.7 ($O\underline{C}H_2CH_3$) 14.3 ($OCH_2\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 2981, 1756, 1622, 1593, 1502, 1462, 1349, 1291, 1200, 1151, 1134, 1096, 1028, 801, 764, 747 cm^{-1}; MS (ESI +ve) *m/z* 379 ([M + Na]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{14}H_{13}O_3NaI$ 378.9807, found 378.9801 ([M + Na]$^+$).

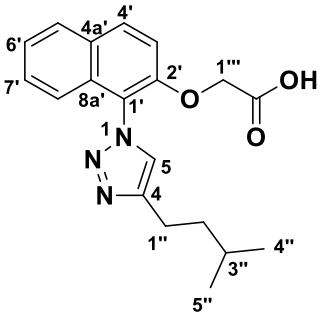
Ethyl 2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetate (76)



To a stirred solution of ethyl 2-(2-iodophenoxy)acetate **75** (0.20 g, 0.54 mmol), 5-methyl-1-hexyne (0.16 g, 1.64 mmol), CuI (0.02 g, 0.11 mmol), NaN_3 (0.04 g, 0.60 mmol) and sodium ascorbate (0.04 g, 0.22 mmol) in DMSO (2.5 mL) H_2O (0.5 mL) was added racemic *trans*-*N*,*N'*-dimethyl cyclohexane-1,2-diamine (0.016 g, 0.11 mmol) at rt under a nitrogen atmosphere. The reaction mixture was stirred and heated at 75 °C for 16 h. The reaction was cooled to rt and aqueous saturated NH_4Cl solution (3 mL) was added and the mixture was extracted with EtOAc (2 x 25 mL). The combined extracts were washed with water (25 mL), brine (25 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica

gel flash column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 10:90 → 100:0) to afford **76** (0.05 g, 25%) as a yellow waxy solid. TLC (EtOAc/*n*-hexane - 33:67); R_f = 0.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.2 Hz, 1H, H8'), 7.84 (d, *J* = 6.4 Hz, 1H, H5'), 7.67 (s, 1H, H5), 7.49-7.41 (m, 2H, H6'/H7'), 7.27-7.25 (m, 2H, H3'/H4'), 4.67 (s, 2H, H1'''), 4.22 (q, *J* = 5.6 Hz, 2H, $OC\underline{H}_2CH_3$), 2.89 (t, *J* = 5.6 Hz, 2H, H1''), 1.73-1.67 (m, 3H, H2''/H3''), 1.26 (t, *J* = 5.6 Hz, 3H, $OCH_2OC\underline{H}_3$), 0.99 (d, *J* = 4.0 Hz, 6H, H4''/H5''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5 (C=O), 150.5 (C2'), 148.1 (C8a'), 131.6 (C4), 131.3 (C4a'), 129.5 (C4'), 128.5 (C5'), 127.9 (C7'), 125.3 (C8'), 124.7 (C6'), 122.1 (C5), 121.3 (C3'), 114.3 (C1'), 66.7 (C1'''), 61.6 ($O\underline{C}H_2CH_3$), 38.6 (C2''), 27.9 (C1''), 23.8 (C3''), 22.5 (C4''/C5''; Observed by gHMBC), 14.2 ($OCH_2\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 2954, 2928, 2868, 1748, 1632, 1600, 1513, 1483, 1454, 1430, 1366, 1288, 1206, 1150, 1117, 1087, 1042, 806, 749 cm^{-1}; MS (ESI +ve) *m/z* 390 ($[M+Na]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{21}H_{26}N_3O_3$ 368.1974, found 368.1985 ($[M+H]^+$).

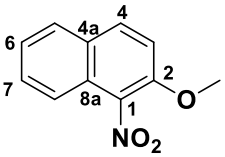
2-((1-(4-Isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetic acid (54)



Following **General Procedure II**, ethyl 2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetate **76** (0.07 g, 0.19 mmol) and 7% KOH solution (0.5 mL) were stirred in ethanol (2 mL) at rt for 2 h to give after acidification the acid **54** (0.04 g, 62%) as a white solid. M.P: 152 – 154 °C. TLC (EtOAc/*n*-hexane - 100:0): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 9.0 Hz, 1H, H8'), 7.88 (d, *J* = 7.5 Hz, 1H, H5'), 7.69 (s, 1H, H5), 7.54-7.46 (m, 2H, H6'/H7'), 7.47-7.29 (m, 2H, H3'/H4'), 4.78 (s, 2H, H1'''), 2.91-2.87 (m, 2H, H1''), 1.71-1.68 (m, 3H, H2''/H3''), 0.98 (d, *J* = 6.0 Hz, 6H, H4''/H5''), COOH resonance was not observed; ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.6 (C=O), 150.4 (C2'), 148.4 (C8a'), 132.2 (C4), 130.7 (C4a'), 129.6 (C4'), 128.8 (C5'), 128.2 (C7'),

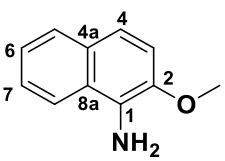
125.6 (C8'), 124.9 (C6'), 121.7 (C5), 120.9 (C3'), 114.4 (C1'), 66.8 (C1'"), 38.5 (C2"), 28.0 (C1"), 23.7 (C3"), 22.6 (C4"/C5"; Observed by gHMBC); IR (neat) $\bar{\nu}_{max}$ 3147, 2954, 2929, 2868, 1731, 1631, 1600, 1514, 1483, 1429, 1366, 1284, 1213, 1151, 1118, 1087, 1062, 923, 806, 748 cm^{-1}; MS (ESI +ve) *m/z* 362 ($[M + Na]^+$, 40%), 340 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{22}N_3O_3$ 340.1661, found 340.1667 ($[M + H]^+$).

2-Methoxy-1-nitronaphthalene (80)[80]



To an ice-cold solution of 2-methoxynaphthalene **79** (3.00 g, 18.96 mmol) in acetic anhydride (35 mL) was added fuming HNO_3 (1.32 g, 20.85 mmol) dropwise at 0 °C. The mixture was stirred at rt for 1 h and placed in a freezer for 16 h. The deposited crystals were filtered, washed with acetic anhydride, water and dried under vacuum for 3 h to afford nitro compound **80** (0.86 g, 22%) as a yellow solid. TLC (EtOAc/*n*-hexane - 20:80): R_f = 0.5; The spectroscopic data were found to be in agreement with that previously reported.[80] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (d, *J* = 8.2 Hz, 1H, H8), 7.84 (d, *J* = 9.1 Hz, 1H, H4), 7.68 (dd, *J* = 8.5, 0.8 Hz, 1H, H5), 7.61-7.57 (m, 1H, H7), 7.47-7.43 (m, 1H, H6), 7.34 (d, *J* = 9.1 Hz, 1H, H3), 4.03 (s, 3H, OMe); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.6 (C2), 132.1 (C8a), 129.1 (C4), 128.2 (C4a), 128.1 (C7), 128.0 (8), 125.6 (C6), 125.1 (C1), 120.4 (C5), 113.0 (C3), 57.0 (OMe); IR (neat) $\bar{\nu}_{max}$ 3030, 2944, 2846, 1636, 1602, 1515, 1459, 1436, 1357, 1280, 1259, 1220, 1155, 1079, 809, 795 cm^{-1}; MS (ESI +ve) *m/z* 204 ($[M + H]^+$, 100%).

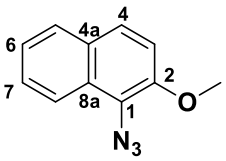
2-Methoxynaphthalen-1-amine (81)[81]



Iron powder (0.14 g, 0.49 mmol) was added to a stirred solution of 2-methoxy-1-nitronaphthalene **80** (0.10 g, 0.49 mmol) in EtOH:H_2O:AcOH

(2 mL : 1 mL : 2 mL) at rt and the mixture was sonicated for 2 h. The reaction was basified with aqueous KOH solution (3 mL) and extracted with EtOAc (2 x 25 mL). The combined extracts were washed with water (25 mL), brine (25 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 10:90 → 100:0) to afford **81** (0.07 g, 82%) as a brown oil. TLC (EtOAc/*n*-hexane - 20:80); R_f = 0.5; The spectroscopic data were found to be in agreement with those previously reported.[81] ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76-7.73 (m, 2H, H8/H5), 7.43-7.39 (m, 1H, H7), 7.33-7.29 (m, 2H, H4/H6), 7.22 (d, *J* = 8.8 Hz, 1H, H3), 4.22 (brs, 2H, NH_2), 3.95 (s, 3H, OMe); ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.5 (C2), 129.5 (C4), 129.4 (C4a), 128.3 (C8a), 125.0 (C5), 123.9 (C7), 123.5 (C8), 120.2 (C1), 118.4 (C6), 113.5 (C3), 56.7 (OMe); IR (neat) $\bar{\nu}_{max}$ 3452, 3366, 2936, 2837, 1683, 1609, 1513, 1475, 1407, 1268, 1242, 1097, 1047, 794, 744 cm^{-1}; MS (ESI +ve) *m/z* 174 ([M + H]$^+$, 60%), 142 (60%), 91 (100%).

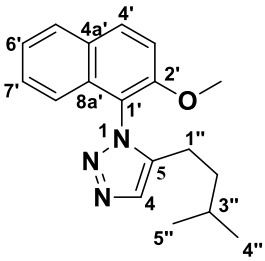
1-Azido-2-methoxynaphthalene (82)



A solution of $NaNO_2$ (0.04 g, 0.60 mmol) in H_2O (0.5 mL) was added dropwise to a stirred suspension of 2-methoxynaphthalen-1-amine **81** (0.07 g, 0.40 mmol) in 3 N HCl solution (2 mL) at 0 °C and stirring at 0 °C was continued for 15 min. Then NaN_3 (0.05 g, 0.80 mmol) in H_2O (0.5 mL) was added at 0 °C and after 10 min the mixture was continued stirring at rt for 3 h. The reaction was diluted with water (10 mL) and extracted with EtOAc (2 x 20 mL). The combined extracts were washed with water (20 mL), brine (20 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 5:95 → 100:0) to afford **82** (0.025

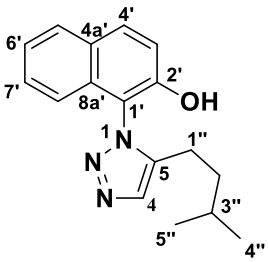
g, 31%) as a brown waxy solid. TLC (EtOAc/*n*-hexane – 20:80); R_f = 0.7; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dd, *J* = 8.5, 1.0 Hz, 1H, H8), 7.72 (d, *J* = 8.2 Hz, 1H, H5), 7.62 (d, *J* = 9.0 Hz, 1H, H4), 7.47-7.43 (m, 1H, H7), 7.37-7.33 (m, 1H, H6), 7.23 (d, *J* = 9.0 Hz, 1H, H3), 4.01 (s, 3H, OMe); ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.3 (C2), 129.3 (C8a), 127.7 (C4), 127.5 (C4a), 126.5 (C5), 125.6 (C8), 124.4 (C7), 122.4 (C1), 121.4 (C6), 114.0 (C3), 57.1 (OMe); IR (neat) $\bar{\nu}_{max}$ 3058, 3005, 2938, 2841, 2103, 2045, 1591, 1507, 1441, 1375, 1290, 1146, 1084, 1017, 912, 806, 747 cm^{-1}; MS (ESI +ve) *m/z* 172 ([M + H – N_2]$^+$, 100%).

5-Isopentyl-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole (83)



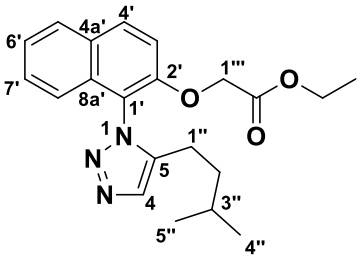
Following **General Procedure VI**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 5-methyl-1-hexyne (0.03 g, 0.27 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 3 h to give the triazole **83** (0.06 g, 81%) as a pale brown waxy solid after flash chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80). TLC (EtOAc/*n*-hexane - 20:80): R_f = 0.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 9.1 Hz, 1H, H8'), 7.85 (dd, *J* = 7.4, 2.0 Hz, 1H, H5'), 7.71 (s, 1H, H4), 7.45-7.37 (m, 3H, H6', H7' and H4'), 6.97 (d, *J* = 9.0 Hz, 1H, H3'), 3.87 (s, 3H, OMe), 2.45-2.34 (m, 2H, H1"), 1.47-1.35 (m, 3H, H2" and H3"), 0.73 (d, *J* = 7.9 Hz, 3H, H5"), 0.72 (d, *J* = 7.9 Hz, 3H, H4"); ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.7 (C2'), 140.7 (C5), 132.0 (C8a'), 131.6 (C4), 131.4 (C4a'), 128.7 (C4'), 128.3 (C5'), 127.9 (C7'), 124.6 (C8'), 121.2 (C6'), 117.9 (C3'), 113.1 (C1'), 56.5 (OMe), 36.7 (C2"), 27.2 (C1"), 22.08 (C5"), 22.07 (C4"), 20.8 (C3"); IR (neat) $\bar{\nu}_{max}$ 2954, 2868, 1630, 1599, 1507, 1457, 1275, 1255, 1064, 1040, 809, 748 cm^{-1}; MS (ESI +ve) *m/z* 318 ([M + Na]$^+$, 50%), 296 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{18}H_{22}N_3O$ 296.1763, found 296.1750 ([M + H]$^+$).

1-(5-Isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-ol (84)



BBr_3 (0.13 g, 0.51 mmol) was added dropwise to a stirred solution of 5-isopentyl-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole **83** (0.05 g, 0.17 mmol) in CH_2Cl_2 (1 mL) at -78 °C and after 30 min the mixture was allowed to stir at rt for 36 h. The reaction was quenched with MeOH (0.5 mL), diluted with EtOAc (25 mL), washed with water (25 mL), brine (25 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80 → 100:0) to afford alcohol **84** (0.04 g, 82%) as a pale brown solid. M.P: 164 - 166 °C. TLC (EtOAc/*n*-hexane – 40:60); R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.31 (brs, 1H, OH), 7.78-7.74 (m, 2H, H8'/H5'), 7.62 (s, 1H, H4), 7.44 (d, *J* = 8.5 Hz, 1H, H4'), 7.38-7.29 (m, 2H, H7'/H6'), 6.84 (d, *J* = 8.5 Hz, 1H, H3'), 2.52-2.32 (m, 2H, H1''), 1.42-1.24 (m, 3H, H2''/H3''), 0.69 (d, *J* = 6.2 Hz, 3H, H5''), 0.65 (d, *J* = 6.2 Hz, 3H, H4''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.6 (C2'), 141.5 (C5), 131.9 (C8a'), 131.4 (C4), 131.2 (C4a'), 128.3 (C4'), 128.0 (C5'), 127.9 (C7'), 123.8 (C8'), 120.4 (C6'), 119.1 (3'), 115.0 (C1'), 36.5 (C2''), 27.2 (C1''), 22.0 (C4''/C5''), 20.8 (C3''); IR (neat) $\bar{\nu}_{max}$ 3061, 2957, 2869, 1638, 1583, 1512, 1440, 1360, 1287, 1245, 1149, 997, 951, 818, 749 cm^{-1}; MS (ESI +ve) *m/z* 304 ([M + Na]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{17}H_{20}N_3O$ 282.1606, found 282.1602 ([M + H]$^+$).

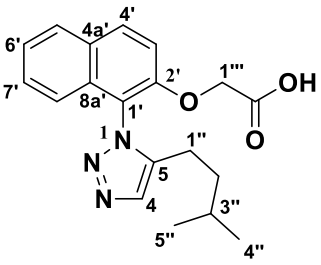
Ethyl 2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetate (85)



Following **General Procedure I**, 1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-ol **84** (0.04 g, 0.14 mmol), K_2CO_3 (0.06 g, 0.42 mmol) and ethyl bromoacetate (0.03 g, 0.18 mmol) were stirred in DMF (1 mL) at rt for 16 h to give the

ester **85** (0.03 g, 58%) as a pale brown oil after flash chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80). TLC (EtOAc/*n*-hexane - 40:60): R_f = 0.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, *J* = 7.2 Hz, 1H, H8'), 7.86 (dd, *J* = 7.3, 2.0 Hz, 1H, H5'), 7.71 (s, 1H, H4), 7.46-7.40 (m, 2H, H6'/H7'), 7.24 (d, *J* = 8.5 Hz, 1H, H4'), 7.02 (d, *J* = 8.5 Hz, H3'), 4.66 (ABq, *J* = 19.0 Hz, 2H, H1'''), 4.22 (q, *J* = 5.6 Hz, 2H, $OC\underline{H}_2CH_3$), 2.63-2.55 (m, 1H, H1''), 2.44-2.38 (m, 1H, H1''), 1.48-1.41 (m, 3H, H2''/H3''), 1.24 (t, *J* = 5.6 Hz, 3H, $OCH_2C\underline{H}_3$), 0.75 (d, *J* = 5.1 Hz, 3H, H5''), 0.72 (d, *J* = 5.0 Hz, 3H, H4''); ^{3}C NMR (101 MHz, $CDCl_3$) δ 168.2 (C=O), 151.0 (C2'), 141.2 (C5), 131.9 (C8a'), 131.6 (C4), 131.4 (C4'), 129.3 (C4a'), 128.4 (C5'), 127.9 (C7'), 125.1 (C8'), 121.5 (C6'), 118.9 (C3'), 113.8 (C1'), 66.2 (C1'''), 61.5 ($O\underline{C}H_2CH_3$), 36.6 (C2''), 27.3 (C1''), 22.1 (C5''), 22.0 (C4''), 20.8 (C3''), 14.1 ($OCH_2\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 2956, 2930, 2869, 1752, 1631, 1600, 1512, 1483, 1292, 1202, 1152, 1095, 1025, 808, 749 cm^{-1}; MS (ESI +ve) *m/z* 390 ($[M + Na]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{21}H_{26}N_3O_3$ 368.2356, found 368.2363 ($[M + H]^+$).

2-((1-(5-Isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetic acid (54)

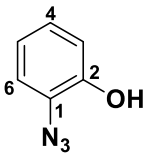


Following **General Procedure II**, ethyl 2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetate **85** (0.28 g, 0.76 mmol) and 7% KOH solution (3 mL) were stirred in ethanol (3 mL) at rt for 2 h to give the acid **54** (0.22 g, 85%) as a white solid. M.P: 148 - 150 °C. TLC (EtOAc/*n*-hexane - 100:0): R_f = 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (d, *J* = 9.0 Hz, 1H, H8'), 7.88-7.86 (m, 1H, H5'), 7.75 (s, 1H, H4), 7.49-7.42 (m, H6'/H7'), 7.30 (d, *J* = 8.5 Hz, 1H, H4'), 7.01 (d, *J* = 8.5 Hz, 1H, H3'), 4.72 (s, 2H, H1'''), 4.59 (brs, 1H, COOH), 2.57-2.49 (m, 1H, H1''), 2.44-2.37 (m, 1H, H1''), 1.46-1.33 (m, 3H, H2''/H3''), 0.72 (d, *J* = 6.2 Hz, 3H, H5''), 0.70 (d, *J* = 6.1 Hz, 3H, H4''); ^{13}C NMR (101 MHz,

$CDCl_3$) δ 170.4 (C=O), 151.1 (C2'), 141.6 (C5), 132.3 (C8a'), 131.2 (C4), 131.2 (4'), 129.3 (C4a'), 128.6 (C5'), 128.1 (C7'), 125.2 (C8'), 121.3 (C6'), 118.6 (3'), 113.9 (C1'), 66.2 (C1'"), 36.5 (C2"), 27.2 (C1"), 22.0 (C4"/C5"), 20.9 (C3"); IR (neat) $\bar{\nu}_{max}$ 2956, 2935, 2869, 1738, 1631, 1601, 1513, 1484, 1428, 1277, 1218, 1152, 1093, 811, 751 cm^{-1}; MS (ESI +ve) *m/z* 362 ($[M + Na]^+$, 30%), 340 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{22}N_3O_3$ 340.1661, found 340.1666 ($[M + H]^+$).

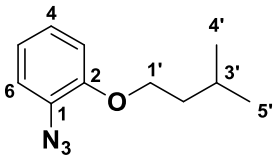
6.3.1.3 – Alternate phenyl triazole acids

2-Azidophenol (87)[79]



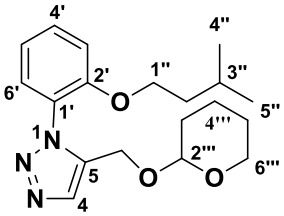
A solution of $NaNO_2$ (5.06 g, 73.39 mmol) in H_2O (5 mL) was added dropwise to a stirred suspension of 2-aminophenol **86** (4.00 g, 36.69 mmol) in 3 N HCl solution (50 mL) at 0 °C and stirring was continued at 0 °C for 15 min. A solution of NaN_3 (4.77 g, 73.39 mmol) in H_2O (5 mL) was added at 0 °C and after 10 min the mixture was stirred at rt for 3 h. The reaction was diluted with water (50 mL) and extracted with EtOAc (2 x 100 mL). The combined extracts were washed with water (100 mL), brine (100 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 10:90 → 100:0) to afford **87** (3.10 g, 60%) as a brown waxy solid. TLC (EtOAc/*n*-hexane – 20:80); R_f = 0.5; The spectroscopic data were found to be in agreement with those previously reported.[79] ^1H NMR (500 MHz, $CDCl_3$) δ 7.07-7.02 (m, 2H, H4/H6), 6.94-6.90 (m, 2H, H3/H5), 5.39 (brs, 1H, OH); ^{13}C NMR (126 MHz, $CDCl_3$) δ 147.1 (C2), 125.8 (C1), 125.8 (C4), 121.1 (C6), 118.2 (C5), 115.9 (C3); IR (neat) $\bar{\nu}_{max}$ 3341, 2121, 1586, 1576, 1496, 1297, 1206, 852, 747 cm^{-1}.

1-Azido-2-(isopentyloxy)benzene (88)



Following **General Procedure I**, 2-azidophenol **87** (2.00 g, 14.80 mmol), K_2CO_3 (6.13 g, 44.40 mmol) and 1-bromo-3-methylbutane (2.46 g, 16.28 mmol) were stirred in dry DMF (16 mL) at rt for 48 h to give compound **88** (2.56 g, 84%) as a brown oil after flash chromatography over SiO_2 gel (EtOAc/*n*-hexane-10:90). TLC (EtOAc/*n*-hexane-20:80): R_f = 0.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05-7.02 (m, 1H, H4), 6.92 (dd, *J* = 8.2, 1.8 Hz, 1H, H6), 6.87-6.84 (m, 2H, H3/H5), 4.01 (t, *J* = 6.7 Hz, 2H, H1'), 1.88-1.82 (m, 1H, H3'), 1.71 (q, *J* = 6.7 Hz, 2H, H2'), 0.96 (d, *J* = 6.6 Hz, 6H, H4'/H5'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.0 (C2), 128.3 (C1), 125.7 (C6), 121.4 (C4), 120.8 (C5), 112.9 (C3), 67.4 (C1'), 37.8 (C2'), 25.1 (C3'), 22.6 (C4'/C5'); IR (neat) $\bar{\nu}_{max}$ 2956, 2871, 2107, 1592, 1495, 1451, 1386, 1304, 1239, 1149, 1042, 980, 741 cm^{-1}; MS (ESI +ve) *m/z* 178 ($[M - N_2 + H]^+$, 100%).

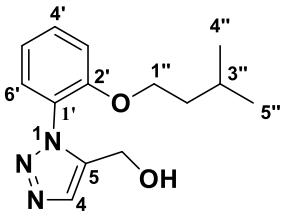
1-(2-(Isopentyloxy)phenyl)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-1*H*-1,2,3-triazole (89)



Following **General Procedure VI**, 1-azido-2-(isopentyloxy)benzene **88** (1.30 g, 6.34 mmol) was treated with tetrahydro-2-(2-propynyloxy)-2*H*-pyran (0.98 g, 6.97 mmol) and EtMgCl (0.67 g, 7.61 mmol; 2 M in Et_2O) in dry THF (13 mL) at 50 °C for 48 h to give triazole **89** (1.20 g, 55%) as a pale brown oil after flash chromatography over SiO_2 gel (EtOAc/*n*-hexane - 10:90). TLC (EtOAc/*n*-hexane - 20:80): R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (s, 1H, H4), 7.48-7.44 (m, 1H, H4'), 7.39 (dd, *J* = 7.9, 1.7 Hz, 1H, H6'), 7.08-7.05 (m, 2H, H3'/H5'), 4.69 (d, *J* = 13.1 Hz, 1H, C$\underline{H}_A$H$_B$OTHP), 4.52 (t, *J* = 3.2 Hz, 1H, H2'''), 4.46 (d, *J* = 13.1 Hz, 1H, CH$_A\underline{H}_B$OTHP), 3.98 (t, *J* = 6.5 Hz, H1''), 3.56-3.52 (m, 1H, H6'''), 3.40-3.37 (m, 1H, H6'''),

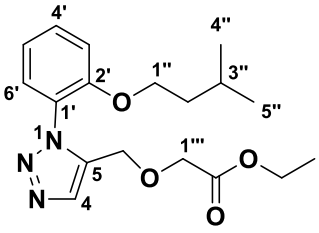
1.69-1.64 (m, 1H, H3''), 1.61-1.37 (m, 8H, H2''/H3'''/H4'''/H5'''), 0.83 (d, J = 6.4 Hz, 6H, H4''/H5''); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.5 (C2'), 136.5 (C5), 132.8 (C4), 131.5 (C4'), 128.6 (C6'), 125.4 (C1'), 120.7 (C5'), 113.1 (C3'), 97.8 (C2'''), 67.5 (C1''), 61.6 (C6'''), 57.9 ($\underline{C}H_AH_B$OTHP), 37.6 (C2''), 30.1 (C3'''), 25.3 (C5'''), 25.0 (C3''), 22.5 (C4''), 22.4 (C5''), 18.7 (C4'''); IR (neat) $\bar{\nu}_{max}$ 2952, 2871, 1602, 1509, 1465, 1387, 1350, 1287, 1261, 1247, 1123, 1056, 1033, 975, 871, 816, 755 cm^{-1}; MS (ESI +ve) *m/z* 346 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{27}N_3O_3Na$ 368.1950, found 368.1964 ([M + Na]$^+$).

(1-(2-(Isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methanol (90)



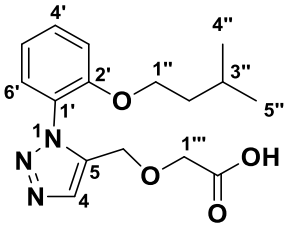
To a stirred solution of 1-(2-(isopentyloxy)phenyl)-5-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)-1*H*-1,2,3-triazole **89** (1.17 g, 3.39 mmol) in MeOH (15 mL) was added *p*-toluene sulfonic acid monohydrate (0.71 g, 3.73 mmol) at rt and the mixture was allowed to stir at rt for 16 h. The reaction mixture was concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel (MeOH/CH_2Cl_2 - 5:95 → 100:0) to afford **90** (0.71 g, 80%) as a light brown oil. TLC (MeOH/CH_2Cl_2 – 10:90); R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (s, 1H, H4), 7.50-7.46 (m, 1H, H4'), 7.37-7.35 (m, 1H, H6'), 7.09-7.06 (m, 2H, H3'/H5'), 4.58 (s, 2H, C$\underline{H}_2$OH), 3.99 (t, J = 6.6 Hz, 2H, H1''), 3.95 (brs, 1H, OH), 1.55-1.47 (m, 3H, H2''/H3''), 0.81 (d, J = 6.4 Hz, 6H, H4''/H5''); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.2 (C2'), 139.4 (C5), 132.3 (C4), 131.8 (C4'), 128.7 (C6'), 125.0 (C1'), 121.2 (C5'), 113.5 (C3'), 67.9 (C1''), 53.9 ($\underline{C}H_2$OH), 37.5 (C2''), 25.0 (C3''), 22.5 (C4''/C5''); IR (neat) $\bar{\nu}_{max}$ 2955, 2871, 1602, 1508, 1464, 1387, 1368, 1287, 1244, 1164, 1127, 1099, 1048, 1019, 978, 831, 756, 660 cm^{-1}; MS (ESI +ve) *m/z* 262 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{14}H_{20}N_3O_2$ 262.1569, found 262.1556 ([M + H]$^+$).

Ethyl 2-((1-(2-(isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)acetate (91)



To a stirred solution of (1-(2-(isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methanol **90** (0.25 g, 0.96 mmol) in dry THF (2 mL) at 0 °C under a nitrogen atmosphere was added NaH (0.06 g, 1.43 mmol; 60 %), followed by ethyl bromoacetate (0.21 g, 1.24 mmol). After 10 min the mixture was allowed to stir at rt for 16 h. The reaction was diluted with water (10 mL) and extracted with EtOAc (2 x 25 mL). The combined extracts were washed with water (2 x 25 mL), brine (2 x 25 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum and the residue was subjected to silica gel flash column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80 → 100:0) to afford **91** (0.23 g, 69%) as a brown gummy solid. TLC (EtOAc/*n*-hexane – 40:60); R_f = 0.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H, H4), 7.50-7.47 (m, 1H, H6'), 7.41 (ddd, *J* = 7.7, 7.7, 1.6 Hz, 1H, H4'), 7.11-7.06 (m, 2H, H3'/H5'), 4.62 (s, 2H, C$\underline{H}_2$OCOOEt), 4.16 (q, *J* = 7.1 Hz, 2H, OC$\underline{H}_2$CH$_3$), 3.98 (t, *J* = 6.6 Hz, 2H, H1''), 3.95 (s, 2H, H1'''), 1.56-1.48 (m, 3H, H2''/H3''), 1.24 (t, *J* = 7.1 Hz, 3H, OCH$_2$C$\underline{H}_3$), 0.83 (d, *J* = 6.4 Hz, 6H, H4''/H5''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.0 (C=O), 153.6 (C2'), 135.7 (C5), 133.6 (C4), 132.0 (C4'), 128.8 (C6'), 125.2 (C1'), 121.2 (C5'), 113.4 (C3'), 67.8 (C1'''), 67.5 (C1''), 62.3 ($\underline{C}$H$_2$O), 61.4 (O$\underline{C}$H$_2$CH$_3$), 37.8 (C2''), 25.2 (C3''), 22.8 (C4''/C5''), 14.5 (OCH$_2$$\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 2957, 2872, 1750, 1602, 1509, 1465, 1386, 1369, 1287, 1265, 1247, 1207, 1135, 1048, 1025, 977, 756 cm^{-1}; MS (ESI +ve) *m/z* 370 ([M + Na]$^+$, 70%), 348 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{18}H_{26}N_3O_4$ 348.1923, found 348.1922 ([M + H]$^+$).

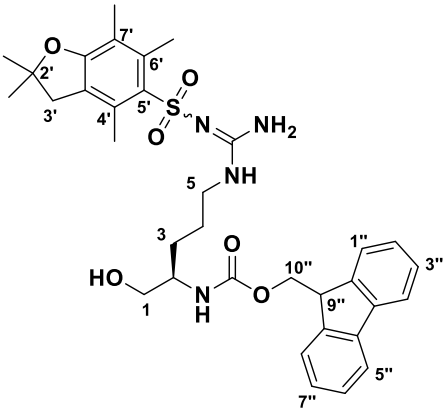
2-((1-(2-(Isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)acetic acid (55)



To a stirred solution of ethyl 2-((1-(2-(isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)acetate **91** (0.23 g, 0.66 mmol) in THF:H_2O (3 mL:1mL) was added LiOH.H_2O (0.14 g, 3.31 mmol) at rt and the reaction was allowed to stir at rt for 16 h. The reaction mixture was diluted with water (5 mL), acidified with 1 M HCl (5 mL) and extracted with EtOAc (2 x 25 mL). The combined organic extracts were washed with brine (50 mL) and dried ($MgSO_4$). The solution was filtered, concentrated under vacuum to afford the acid **55** (0.17 g, 81%) as a pale brown waxy solid. TLC (EtOAc/*n*-hexane – 100:0); R_f = 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (brs, 1H, COOH), 7.88 (s, 1H, H4), 7.50-7.46 (m, 1H, H4'), 7.40 (d, *J* = 7.6 Hz, 1H, H6'), 7.10-7.05 (m, 2H, H3'/H5'), 4.63 (s, 2H, H1'''), 4.00 (s, 2H, CH_2O), 3.98-3.97 (m, 2H, H1''), 1.57-1.47 (m, 3H, H2''/H3''), 0.82 (d, *J* = 6.2 Hz, 6H, H4''/H5''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0 (C=O), 153.3 (C2'), 135.7 (C5), 132.7 (C4), 131.9 (C4'), 128.4 (C6'), 124.6 (C1'), 120.9 (C5'), 113.2 (C3'), 67.6 (C1'''), 67.0 (C1''), 61.9 (C-C5), 37.5 (C2''), 24.9 (C3''), 22.4 (C4''/C5''); IR (neat) $\bar{\nu}_{max}$ 2955, 2931, 2871, 2533, 1733, 1602, 1508, 1464, 1429, 1368, 1287, 1246, 1163, 1116, 1049, 994, 846, 755, 662 cm^{-1}; MS (ESI +ve) *m/z* 342 ([M + Na]$^+$, 100%), 320 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{16}H_{22}N_3O4$ 320.1610, found 320.1603 ([M + H]$^+$).

6.3.2 – Synthesis of *N*-protected β-azido-amines

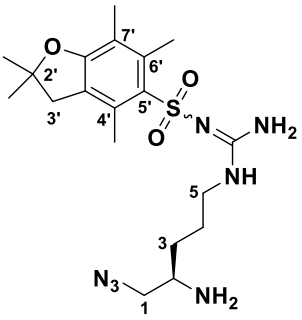
(9*H*-Fluoren-9-yl)methyl (*R*)-(1-hydroxy-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydro benzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)carbamate (93)[77]



Commercial Fmoc-(D)-Arg(Pbf)-OH **92** (10.00 g, 15.41 mmol) was dissolved in THF (80 mL) with magnetic stirring. The solution was cooled to −10 °C (ice/salt bath) followed by sequential dropwise addition of isobutyl chloroformate (2.31 g, 16.95 mmol) and *N*-methylmorpholine (1.70 g, 16.95 mmol). The mixture was stirred at −10 °C for 30 min and filtered through a small pad of Celite, which was rinsed with fresh, cold THF (5 x 20 mL) and the combined filtrates were kept cold (ice bath). The cold filtrate was added dropwise to a cold aqueous solution of $NaBH_4$ (0.90 g, 23.68 mmol in 10 mL H_2O) with vigorous magnetic stirring. After the addition was complete, the mixture was stirred for 5 min and added to H_2O (50 mL) with vigorous stirring. The reaction was stirred for 10 min and then the aqueous mixture was extracted with EtOAc (2 x 200 mL). The combined organic extracts were washed with H_2O (2 x 200 mL), brine (200 mL), dried ($MgSO_4$), filtered and concentrated. The residue was subjected to flash chromatography over SiO_2 gel to afford the alcohol **93** (8.10 g, 82%) as a translucent gum. The spectroscopic data were found to be in agreement with those previously reported.[77] TLC (MeOH/CH_2Cl_2 – 10:90): R_f = 0.7; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 (d, *J* = 7.7 Hz, 2H, H4"/H5"), 7.54 (d, *J* = 7.5 Hz, 2H, H1"/H8"), 7.34 (t, *J* = 7.4 Hz, 2H, H3"/H6"), 7.25-7.20 (m, 2H, H2"/H7"), 6.32-6.05 (m, 3H, N^5-H/NH_2 (guanidine)), 5.67-5.51 (brs, 1H, N^2-H), 4.34 (d, *J* = 7.0 Hz, 2H, H10"), 4.13 (t, *J* = 7.0 Hz, 1H, H9"), 3.71-3.47 (m, 3H, H1/H2), 3.25-3.14 (m, 2H, H5), 2.89 (s, 2H, H3'), 2.56 (s, 3H, C6'-C$\underline{H}_3$), 2.49 (s, 3H, C4'-C$\underline{H}_3$), 2.06 (s, 3H, C7'-C$\underline{H}_3$), 1.65-1.45

(m, 4H, H3/H4), 1.41 (s, 6H, C2'-(C$\underline{H}_3$)$_2$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.9 (C7a'), 157.0 (C=O), 156.3 (C=N), 143.9 (C1a"/C8a"), 143.8 (C4a"), 141.3 (C5a"), 138.4 (C3a'), 132.6 (C6'), 132.3 (C4'), 127.7 (C5'), 127.1 (C3"/C6"), 125.1 (C2"/C7"), 124.7 (C4"/C5"), 119.9 (C1"/ C8"), 117.6 (C7'), 86.5 (C2'), 66.7 (C10"), 64.7 (C1), 52.5 (C2), 47.2 (C9"), 43.2 (C3'), 40.0 (C5), 28.6 (C3, C2'-(C$\underline{H}_3$)$_2$), 25.6 (C4), 19.3 (C4'-C$\underline{H}_3$), 18.0 (C6'-C$\underline{H}_3$), 12.5 (C7'-C$\underline{H}_3$); MS (ESI +ve) *m/z* 657 ($[M + Na]^+$, 100%).

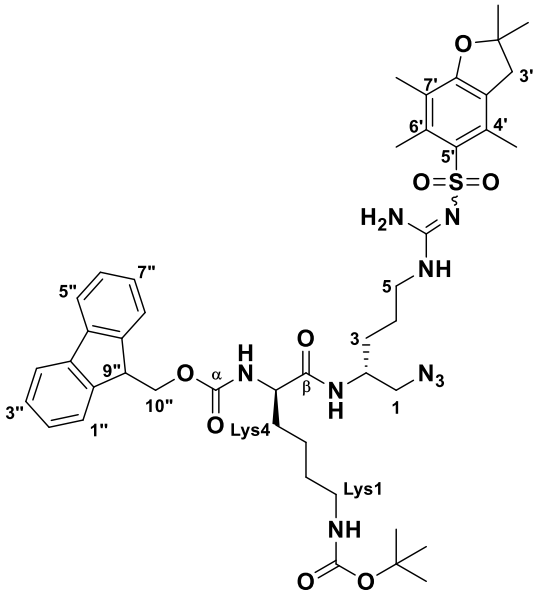
(*R*)-*N*-(Amino((4-amino-5-azidopentyl)amino)methylene)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamide (56)[47]



A solution of triphenylphosphine (1.82 g, 6.94 mmol), iodine (1.76 g, 6.94 mmol), imidazole (0.52 g, 7.57 mmol) in CH_2Cl_2 (15 mL) was stirred at rt for 10 min followed by the addition of a solution of the alcohol **93** (4.00 g, 6.31 mmol) in CH_2Cl_2 (5 mL). The reaction was stirred at rt for 25 h, diluted with CH_2Cl_2 (50 mL) and filtered to remove the white precipitate. The filtrate was concentrated, and the residue was subjected to flash chromatography over SiO_2 gel (EtOAc/Et_2O – 50:50) to give a mixture of the intermediate iodide and triphenylphosphine oxide. The mixture was dissolved in DMF (25 mL) and NaN_3 (2.05 g, 31.51 mmol) was added to the solution followed by vigorous stirring at rt for 4 h. After the azidation reaction was shown to be complete by TLC analysis (EtOAc/*n*-hexane. – 80:20), the reaction was heated at 50 °C (oil bath) overnight (12 h) to remove the *N*-Fmoc protecting group. The reaction mixture was cooled to rt and then partitioned between EtOAc (200 mL) and aqueous HCl (0.5 M – 200 mL) with magnetic stirring for 5 min. The acidic aqueous phase was separated and washed with EtOAc (3 x 100 mL). The aqueous phase was made alkaline (pH > 10) with aqueous NaOH solution (10%

w/w) and was extracted with EtOAc (3 x 100 mL). The organic extracts were combined and washed with H_2O (100 mL), brine (100 mL), dried ($MgSO_4$), filtered and concentrated to afford the amine **56** (2.00 g, 73% over two steps) as an off-white foam that collapsed into a translucent, pale yellow gum. The spectroscopic data were found to be in agreement with those previously reported.[47] TLC (MeOH/CH_2Cl_2 – 10:90): R_f = 0.21; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.38-6.08 (m, 3H, N^1-H/NH_2 (guanidine)), 3.37 (m, 1H, $H1_A$ or $H1_B$), 3.27-3.09 (m, 3H, H5, $H1_A$ or $H1_B$), 2.95 (s, 2H, H3'), 2.93-2.87 (m, 1H, H2), 2.58 (s, 3H, C4'-C$\underline{H}_3$), 2.51 (s, 3H, C6'-C$\underline{H}_3$), 2.10 (s, 3H, C7'-C$\underline{H}_3$), 1.73 (s, 2H, -NH_2), 1.71-1.58 (m, 2H, H4), 1.58-1.48 (m, 1H, $H3_A$ or $H3_B$), 1.46 (s, 6H, C2'-(C$\underline{H}_3$)$_2$), 1.42-1.29 (m, 1H, $H3_A$ or $H3_B$); ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.8 (C7a'); 156.2 (C=N), 138.3 (C3a'), 132.9 (C6'), 132.3 (C4'), 124.7 (C5'), 117.5 (C7'), 86.4 (C2'), 58.3 (C1), 50.8 (C2), 43.3 (C3'), 41.0 (C5), 31.0 (C3), 28.6 (C2'-(C$\underline{H}_3$)$_2$), 25.8 (C4), 19.3 (C4'-C$\underline{H}_3$), 17.9 (C6'-C$\underline{H}_3$), 12.5 (C7'-C$\underline{H}_3$); MS (ESI +ve) *m/z* 460 ([M + Na]$^+$, 20%), 438 ([M + H]$^+$, 100%).

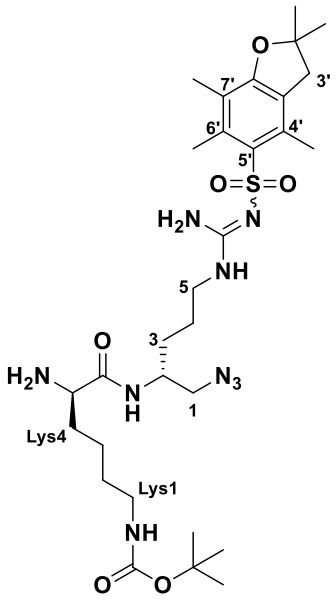
(9*H*-Fluoren-9-yl)methyl *tert*-butyl ((*R*)-6-(((*R*)-1-azido-5-(2-((2,2-dimethyl-2,3-dihydro benzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-6-oxohexane-1,5-diyl) dicarbamate (95).



To a reaction vessel charged with azide **56** (1.38 g, 3.16 mmol), Fmoc-L-Lys(Boc)-OH **94** (1.62 g, 3.50 mmol), EDCI (0.67 g, 3.50 mmol) and HOBt (0.53 g, 3.50 mmol) was added CH_2Cl_2 (10 mL) and the mixture was stirred at rt for 12 h. The reaction mixture was concentrated and

diluted with water (100 mL) and extracted with EtOAc (3 x 100 mL). The organic extracts were combined and washed with HCl (1 M – 100 mL), aqueous $NaHCO_3$ (100 mL), brine (25 mL), dried ($MgSO_4$) and concentrated to give a pale-yellow residue. This residue was purified *via* flash chromatography over SiO_2 (MeOH/ CH_2Cl_2 = 4:96) to afford **95** as an off-white foam (1.50 g, 54%). TLC (MeOH/CH_2Cl_2 – 10:90) R_f = 0.52; ^{1}H-NMR (400 MHz, $CDCl_3$**)** δ 7.77-7.70 (m, 2H, H4"/H5"), 7.55 (d, *J* = 7.5 Hz, 2H, H1"/H8"), 7.55 (brs, 1H, βCONH), 7.41-7.32 (m, 2H, H2"/H7"), 7.29-7.21 (m, 2H, H3"/H6"), 7.17 (brs, 1H, αCONH), 6.31-6.24 (m, 2H, NH_2 (guanidine)), 6.19-6.09 (brs, 1H, N^5-H), 4.82-4.72 (brs, 1H, LysN^1-H), 4.33 (d, *J* = 7.4 Hz, 2H, H10"), 4.25-4.07 (m, 2H, Lys5/H9"), 4.07-3.97 (m, 1H, H2), 3.41-3.23 (m, 2H, H1), 3.23-2.98 (m, 4H, H5/Lys1), 2.89 (s, 2H, H3'), 2.55 (s, 3H, C6'-C$\underline{H}_3$), 2.48 (s, 3H, C4'-C$\underline{H}_3$), 2.06 (s, 3H, C7'-C$\underline{H}_3$), 1.67 (s, 6H, C2'-C$\underline{H}_3$), 1.55-1.35 (m, 19H, H3/H4/Lys2/ Lys3/Lys4/C(C$\underline{H}_3)_3$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7 (Cβ), 158.8 (C7a'), 156.7 (Cα), 156.4 (C=N), 156.2 ($\underline{C}OOC(CH_3)_3$), 143.85 (C1a"or C8a"), 143.83 (C8a" or C1a"), 143.82 (C4a" or C5a"), 143.6 (C5a" or C4a"), 138.3 (C3a'), 132.8 (C6'), 132.2 (C4'), 127.8 (C3"/C6"), 127.1 (C4"/C5"), 125.0 (C2"/C7"), 124.7 (C5'), 120.0 (C1"/C8"), 117.6 (C7'), 86.4 (C2'), 79.3 ($\underline{C}(CH_3)_3$), 67.3 (C10"), 55.1 (Lys5), 54.8 (C1), 48.8 (C2), 47.0 (C9"), 43.2 (C3'), 40.9 (C5), 39.9 (Lys1), 31.9 (Lys2), 29.5 (Lys4), 29.3 (C3), 28.6 (C2'-($\underline{C}H_3)_2$), 28.4 (C($\underline{C}H_3)_3$), 25.5 (C4), 22.5 (Lys3), 19.3 (C6'-$\underline{C}H_3$), 17.9 (C4'-$\underline{C}H_3$), 12.5 C7'-$\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 3322, 2101, 1634, 1548, 1450, 1248, 1165, 1092, 739, 567 cm^{-1}; MS (ESI +ve) *m/z* 910 ([M + Na]$^+$, 80%), 888 ([M + H]$^+$, 100%),; HRMS (ESI +ve TOF) calcd for $C_{45}H_{61}N_9O_8SNa$ 910.4262, found 910.4218 ([M + Na]$^+$).

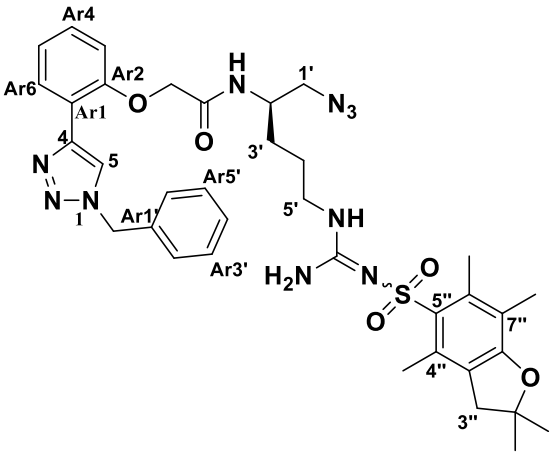
***Tert*-butyl ((*R*)-5-amino-6-(((*R*)-1-azido-5-(2-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-6-oxohexyl)carbamate (57).**



To a solution of Fmoc-protected amine **95** (1.50 g, 1.69 mmol) in acetonitrile (15 mL) was added piperidine (0.25 mL, 1.5 eq.) and the reaction was stirred vigorously at rt for 12 h. The reaction mixture was diluted with MeOH (50 mL) and extracted with hexane (50 mL) multiple times until TLC analysis showed no byproduct (dibenzofulvene piperidine adduct) present in the MeOH layer. Then MeOH extract was concentrated under reduced pressure to give **57** as an off-white foam (0.80 g, 71%). TLC (MeOH/CH_2Cl_2 – 10:90) R_f =0.2; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.61 (brs, 1H, N^2-H), 6.42-6.20 (m, 3H, N^5-H/ NH_2 (guanidine)), 4.82-4.72 (m, 1H, LysN^1-H), 4.12-3.99 (m, 1H, Lys5), 3.46-3.29 (m, 3H, H1/H2), 3.29-3.14 (m, 2H, H5), 3.14-3.04 (m, 2H, Lys1), 2.96 (s, 2H, C3'), 2.58 (s, 3H, C6'-C$\underline{H}_3$), 2.52 (s, 3H, C4'-C$\underline{H}_3$), 2.10 (s, 3H, C7'-C$\underline{H}_3$), 1.62-1.31 (m, 25H, H3/H4/Lys2/Lys3/Lys4/C(C$\underline{H}_3$)$_3$/C2'-(C$\underline{H}_3$)$_2$), N^5H_2 resonance was not observed; ^{13}C-NMR (126 MHz, $CDCl_3$) δ 158.8 (C7a'), 156.6 (C=O), 156.4 (C=N), 138.5 (C3a'), 133.2 (C4'), 132.4 (C6'), 124.7 (C5'), 117.6 (C7'), 86.5 (C2'), 79.4 ((C(CH$_3$)$_3$), 55.1 (Lys5), 55.0 (C1), 46.9 (C2), 43.4 (C3'), 40.9 (C5), 40.4 (Lys1), 34.7 (Lys4), 30.1 (Lys2), 29.8 (C3), 28.8 (C2'-(C$\underline{H}_3$)$_2$), 28.6 (C(C$\underline{H}_3$)$_3$), 25.8 (C4), 22.7 (Lys3), 19.4 (C6'-C$\underline{H}_3$), 18.1 (C4'-C$\underline{H}_3$), 12.6 (C7'-C$\underline{H}_3$), COO(C(CH$_3$)$_3$) resonance was not observed; IR (neat) $\bar{\nu}_{max}$ 3327, 2101, 1685, 1620, 1551, 1454, 1366, 1278, 1250, 1168, 1094, 665, 569 cm$^{-1;}$ MS (ESI +ve) *m/z* 688 ([M + Na]$^+$, 20%), 666 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{30}H_{52}N_9O_6S$ 666.3761, found 666.3741 ([M + H]$^+$).

6.3.3 – Synthesis of the *N*-protected β-azides

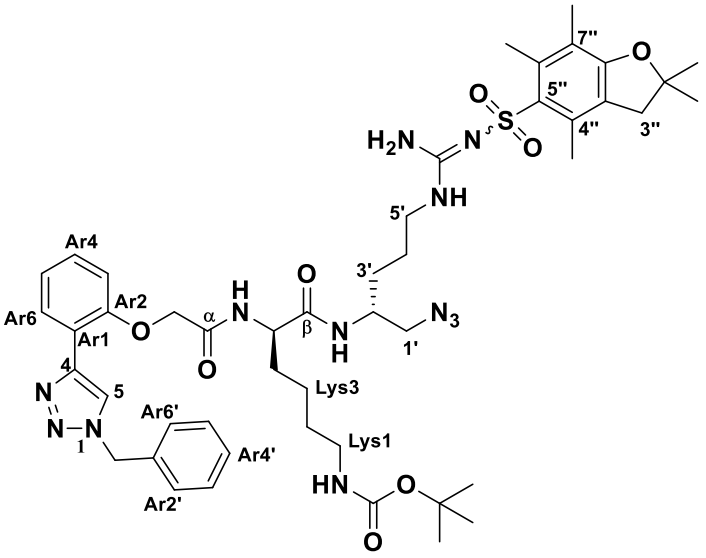
(*R*)-*N*-(1-Azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) guanidino)pentan-2-yl)-2-(2-(1-benzyl-1*H*-1,2,3-triazol-4-yl)phenoxy)acetamide (58)



Following **General Procedure III**, 2-(2-(1-benzyl-1*H*-1,2,3-triazol-4-yl)phenoxy)acetic acid **50** (0.05 g, 0.16 mmol), (*R*)-*N*-(amino ((4-amino-5-azidopentyl)amino)methylene)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamide **56** (0.07 g, 0.16 mmol), EDCI.HCl (0.03 g, 0.17 mmol), HOBt (0.03 g, 0.17 mmol) and TEA (0.01 g, 0.16 mmol) were stirred in CH_2Cl_2 (2 mL) at rt for 16 h to give the acetamide **58** (0.08 g, 69%) as a yellow waxy solid. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 1H, H5), 7.51 (brs, 1H, CONH), 7.35-7.25 (m, 7H, Ar6/Ar4/Ar2'/Ar3'/Ar4'/Ar5'/Ar6'), 7.00 (t, *J* = 7.5 Hz, 1H, Ar5), 6.88 (d, *J* = 8.5 Hz, 1H, Ar3), 6.28-6.20 (m, 3H, N$^{5'}$-H/ NH_2 (guanidine)), 5.55 (ABq, *J* = 15.0 Hz, 2H, OCH_AH_B), 4.61 (s, 2H, CH_2Ph), 4.12-4.08 (m, 1H, H2'), 3.48-3.32 (m, 2H, H1'), 3.24-3.10 (m, 2H, H5'), 2.88 (s, 2H, H3''), 2.52 (s, 3H, C4''-CH_3), 2.45 (s, 3H, C6''-CH_3), 2.70 (s, 3H, C7''-CH_3), 1.80-1.66 (m, 1H, H3'), 1.64-1.40 (m, 3H, H3'/H4'), 1.42 (s, 6H, C2''-$(CH_3)_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.6 (C=O), 158.7 (C7a''), 156.4 (C=N), 154.3 (Ar2), 145.6 (C4), 138.45 (Ar1'), 138.44 (C4''/C6''), 134.6 (C7''), 133.1 (C3a''), 132.3 (C5''), 130.0 (C5), 129.2 (Ar4), 128.9 (Ar3'/Ar5'), 128.2 (Ar2'/Ar6'), 124.7 (Ar6), 122.1 (Ar4'), 119.3 (Ar1), 117.5 (Ar5), 113.3 (Ar3), 86.5 (C2''), 67.9 (OCH_AH_B), 60.5 (CH_2Ph), 54.5 (C1'), 54.3 (C2'), 49.1 (C5'), 43.3 (C3''), 41.0 (C3'), 29.7 (C2''-$(CH_3)_2$), 25.5 (C4'), 19.4 (C4''-CH_3), 18.0 (C6''-CH_3), 12.6 (C7''-CH_3); IR (neat) $\bar{\nu}_{max}$ 3438, 3332, 3227, 3139, 2970, 2867, 2101, 1666, 1619, 1551,

1487, 1454, 1407, 1384, 1370, 1354, 1291, 1262, 1152, 1106, 1093, 1060, 1040, 994, 976, 852, 784, 757, 664, 642 cm^{-1}; MS (ESI +ve) *m/z* 767 ($[M + K]^+$, 25%), 751 ($[M + Na]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{36}H_{44}N_{10}O_5SNa$ 751.3109, found 751.3108 ($[M + Na]^+$).

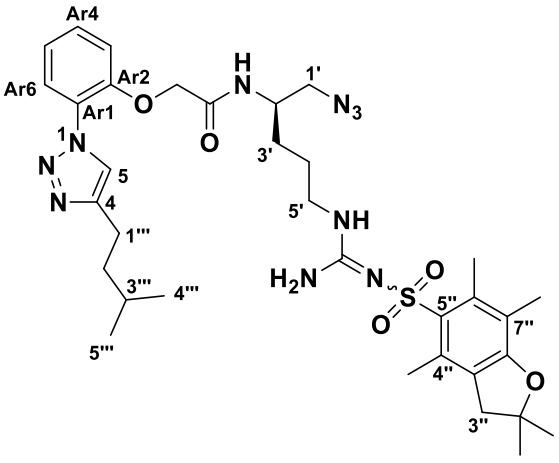
***Tert*-butyl ((*R*)-6-(((*R*)-1-azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-(2-(2-(1-benzyl-1*H*-1,2,3-triazol-4-yl)phenoxy)acetamido)-6-oxohexyl)carbamate (59)**



Following **General Procedure III**, 2-(2-(1-benzyl-1*H*-1,2,3-triazol-4-yl)phenoxy)acetic acid **50** (0.09 g, 0.30 mmol), *tert*-butyl ((*R*)-5-amino-6-(((*R*)-1-azido-5-(2-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) guanidino)pentan-2-yl)amino)-6-oxohexyl)carbamate **57** (0.20 g, 0.30 mmol), EDCI.HCl (0.06 g, 0.33 mmol), HOBt (0.05 g, 0.33 mmol) and TEA (0.03 g, 0.30 mmol) were stirred in CH_2Cl_2 (3 mL) at rt for 16 h to give the acetamide **59** (0.14 g, 49%) as a white solid. M.P: 244 - 246 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (brs, 1H, βCONH), 7.93 (s, 1H, H5), 7.69-7.67 (m, 1H, αCONH), 7.36-7.26 (m, 7H, Ar6/Ar4/Ar2'/Ar3'/Ar4'/Ar5'/Ar6'), 7.11-7.10 (m, 1H, LysN^1-H), 7.04 (t, *J* = 7.5 Hz, 1H, Ar5), 6.92 (d, *J* = 8.5 Hz, 1H, Ar3), 6.28-6.20 (m, 2H, NH_2 (guanidine)), 6.18-6.10 (m, 1H, $N^{5'}$-H (guanidine)), 5.61 (ABq, *J* = 15.0 Hz, 2H, OCH_AH_B), 4.80-4.72 (m, 1H, Lys5), 4.64-4.60 (m, 1H, H1'), 4.64 (s, 2H, CH_2Ph), 4.56-4.46 (m, 1H,

H1'), 4.08-3.98 (m, 1H, H2'), 3.32-3.20 (m, 2H, H5'), 3.20-3.06 (m, 2H, Lys1), 2.92 (s, 2H, H3''), 2.56 (s, 3H, C4''-C$\underline{H}_3$), 2.49 (s, 3H, C6''-C$\underline{H}_3$), 2.07 (s. 3H, C7''-C$\underline{H}_3$), 2.02-1.80 (m, 6H, Lys4/H3'/H4'), 1.48-1.42 (m, 4H, Lys2/Lys3), 1.44 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.40 (s, 6H, C2''-(C$\underline{H}_3$)$_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.6 (βC=O), 169.5 (αC=O), 158.8 (C7a''), 156.4 (C=N), 156.3 (Ar2), 154.1 (C$\underline{O}$OC(CH$_3$)$_3$), 145.0 (C4), 138.4 (Ar1'), 135.0 (C4''), 133.2 (C6''), 132.4 (C3a''), 129.9 (Ar4), 129.3 (C5''), 129.2 (C5), 129.1 (C7''), 129.0 (Ar3'), 128.8 (Ar5'), 128.2 (Ar2'), 124.7 (Ar6'), 122.5 (Ar4'), 122.4 (Ar6), 119.6 (Ar5), 117.6 (Ar3), 113.3 (Ar1), 86.5 (C2''), 79.2 (C$\underline{(CH_3)_3}$), 67.8 (OC$\underline{H}_A$H$_B$), 60.6 (Lys5), 55.0 (C$\underline{H}_2$Ph), 54.3 (C1'), 53.9 (C2'), 53.6 (C5'), 43.4 (C3''), 40.9 (Lys1), 40.2 (Lys4), 31.6 (C3'), 29.5 (Lys2), 28.7 (C(C$\underline{H}_3$)$_3$), 28.6 (C2''-(C$\underline{H}_3$)$_2$), 25.6 (C4'), 23.1 (Lys3), 19.4 (C4''-C$\underline{H}_3$), 18.1 (C6''-C$\underline{H}_3$), 12.6 (C7''-C$\underline{H}_3$); IR (neat) $\bar{\nu}_{max}$ 3426, 3322, 3156, 3065, 2973, 2932, 2865, 2100, 1660, 1619, 1547, 1489, 1454, 1406, 1392, 1366, 1247, 1165, 1105, 1092, 1042, 994, 976, 941, 904, 852, 783, 756, 698, 642 cm$^{-1;}$ MS (ESI +ve) *m/z* 979 ([M + Na]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{47}H_{64}N_{12}O_8SNa$ 979.4583, found 979.4587 ([M + Na]$^+$).

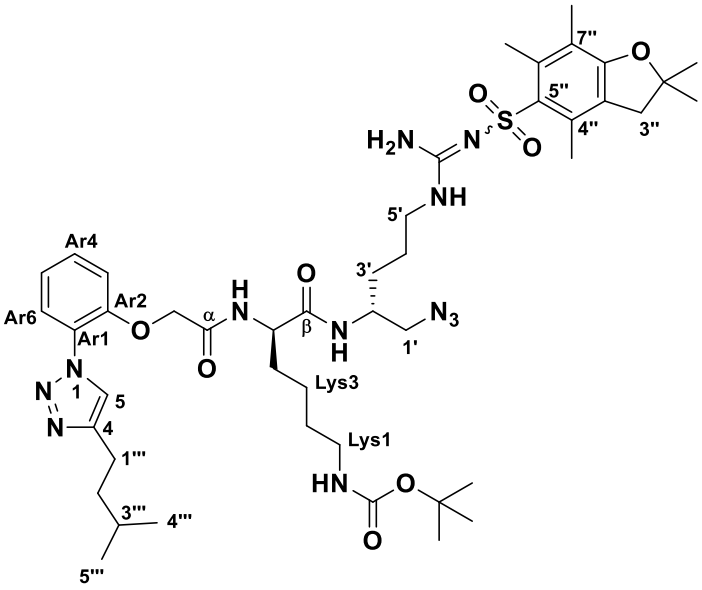
(*R*)-*N*-(1-Azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) guanidino)pentan-2-yl)-2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide (60)



Following **General Procedure III**, 2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetic acid **51** (0.13 g, 0.46 mmol), (*R*)-*N*-(amino ((4-amino-5-azidopentyl)amino)methylene)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamide **56** (0.20 g, 0.46 mmol), EDCI.HCl (0.10 g, 0.50 mmol), HOBt (0.08 g,

0.50 mmol) and TEA (0.05 g, 0.46 mmol) were stirred in CH_2Cl_2 (3 mL) at rt for 14 h to give the acetamide **60** (0.19 g, 58%) as a pale-yellow solid. M.P: 234 - 236 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (s, 1H, H5), 7.42 (t, *J* = 7.5 Hz, 1H, Ar4), 7.39 (d, *J* = 8.0 MHz, 1H, Ar6), 7.22 (brs, 1H, CONH), 7.16 (t, *J* = 7.5 Hz, 1H, Ar5), 7.09 (d, *J* = 8.0 Hz, 1H, Ar3), 6.36 (brs, 1H, $N^{5'}$-H (guanidine)), 6.29 (brs, 2H, NH_2, (guanidine)), 4.61 (ABq, *J* = 9.5 Hz, 2H, OCH_AH_B), 4.08-3.94 (m, 1H, H2'), 3.40-3.30 (m, 2H, H1'), 3.30-3.20 (m, 2H, H5'), 2.94 (s, 2H, H3"), 2.80 (t, *J* = 8.0 Hz, 2H, H1'"), 2.57 (s, 3H, C4"-CH_3), 2.51 (s, 3H, C6"-CH_3), 2.07 (s, 3H, C7"-CH_3), 1.70-1.44 (m, 7H, H2'"/H3'"/H3'/H4'), 1.44 (s, 6H, C2"-$(CH_3)_2$), 0.95 (d, *J* = 6.0 Hz, 6H, H5'"/H4'"); ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.1 (C=O), 158.3 (C7a"), 156.3 (C=N), 151.8 (Ar2), 140.5 (C4"), 138.0 (C6"), 133.1 (C4), 133.0 (C3a"), 132.6 (C5"), 131.4 (Ar4), 127.8 (Ar6), 125.2 (C7"), 124.4 (Ar5), 122.2 (C5), 117.5 (Ar3), 113.3 (Ar1), 86.4 (C2"), 67.8 (OCH_AH_B), 55.2 (C1'), 47.6 (C2'), 43.8 (C2'"), 40.1 (C5'), 36.1 (C3"), 29.9 (C1'"), 29.6 (C3'), 26.1 (C2"-$(CH_3)_2$), 24.5 (C3'"), 22.5 (C4'), 22.4 (C4'"/C5'"), 20.9 (C4"-CH_3), 18.6 (C6"-CH_3), 12.8 (C7"-CH_3); IR (neat) $\bar{\nu}_{max}$ 3430, 3419, 3338, 3332, 3152, 3076, 2956, 2869, 2101, 1680, 1618, 1550, 1508, 1457, 1407, 1385, 1369, 1288, 1252, 1162, 1153, 1106, 1093, 1056, 1038, 994, 978, 852, 815, 784, 760, 667, 642 $cm^{-1;}$ MS (ESI +ve) *m/z* 731 ($[M + Na]^+$, 100%), 709 ($[M + H]^+$, 20%); HRMS (ESI +ve TOF) calcd for $C_{34}H_{48}O_5N_{10}SNa$ 731.3422, found 731.3430 ($[M + Na]^+$).

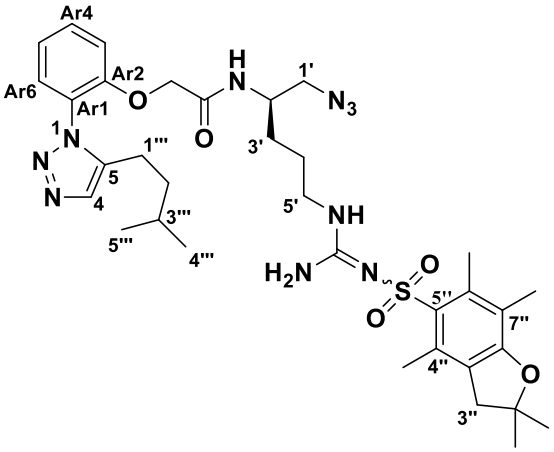
***Tert*-butyl ((*R*)-6-(((*R*)-1-azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-(2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamido)-6-oxohexyl)carbamate (61)**



Following **General Procedure III**, 2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetic acid **51** (0.10 g, 0.34 mmol), *tert*-butyl ((*R*)-5-amino-6-(((*R*)-1-azido-5-(2-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl) sulfonyl)guanidino)pentan-2-yl) amino)-6-oxohexyl)carbamate **57** (0.23 g, 0.34 mmol), EDCI.HCl (0.07 g, 0.38 mmol), HOBt (0.06 g, 0.38 mmol) and TEA (0.03 g, 0.34 mmol) were stirred in CH_2Cl_2 (5 mL) at rt for 16 h to give the acetamide **61** (0.25 g, 78%) as an off-white solid. M.P: 256 - 258 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78-7.74 (m, 1H, Ar6), 7.70 (s, 1H, H5), 7.54-7.36 (m, 3H, Ar4/Ar5/βCONH), 7.20-7.04 (m, 2H, αCONH/Ar3), 6.40-6.10 (m, 3H, $N^{5'}$-H/NH_2 (guanidine)), 5.04-4.96 (m, 1H, LysN^1-H), 4.65-4.56 (m, 2H, OCH_AH_B), 4.44-4.38 (m, 1H, Lys5), 4.04-3.96 (m, 1H, H2'), 3.32-3.24 (m, 2H, H1'), 3.22-2.98 (m, 4H, H5'/Lys1), 2.92 (s, 2H, H3''), 2.84-2.74 (m, 2H, H1'''), 2.54 (s, 3H, C4''-CH_3), 2.48 (s, 3H, C6''-CH_3), 2.06 (s, 3H, C7''-CH_3), 1.90-1.70 (m, 2H, Lys4), 1.64-1.58 (m, 4H, H4'/Lys2), 1.56-1.26 (m, 7H, H3'/ Lys3/H2'''/ H3'''), 1.46 (s, 9H, $C(CH_3)_3$), 1.44 (s, 6H, C2''-$(CH_3)_2$), 0.33 (d, J = 5.5 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.5 (βC=O), 168.1 (αC=O), 158.1 (C7a''), 156.4 (C=N), 150.4 ($COO(CH_3)_3$), 145.9 (Ar2), 138.4 (C4), 132.4 (C4''), 131.1 (C6''), 130.8 (C3a''), 129.6 (C5''), 126.6 (Ar4), 126.4

(C7''), 124.7 (Ar6), 122.6 (Ar5), 117.5 (C5), 117.3 (Ar3), 111.3 (Ar1), 86.4 (C2''), 79.1 ($\underline{C}(CH_3)_3$), 67.4 ($O\underline{C}H_AH_B$), 54.9 (Lys5), 53.7 (C1'), 43.4 (C2'), 40.9 (C2'''), 38.5 (C5'), 31.5 (C3''), 31.3 (Lys1), 29.8 (Lys4), 29.4 (C3'), 28.7 (Lys2), 28.5 (C1'''), 27.85 ($C(\underline{C}H_3)_3$), 27.83 (C2''-$(\underline{C}H_3)_2$), 25.5 (C4'), 23.5 (C3'''), 22.9 (Lys3), 22.5 (C4'''/C5'''), 19.3 (C4''-$\underline{C}H_3$), 18.0 (C6''-$\underline{C}H_3$), 12.5 (C7''-$\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 3316, 3071, 2953, 2932, 2868, 2100, 1662, 1619, 1602, 1546, 1508, 1459, 1420, 1389, 1365, 1250, 1195, 1165, 1109, 1056, 1026, 982, 913, 834, 816, 755, 665, 644, 622 cm^{-1}; MS (ESI +ve) *m/z* 959 ($[M + Na]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{45}H_{69}N_{12}O_8S$ 937.5082, found 937.5098 ($[M+H]^+$).

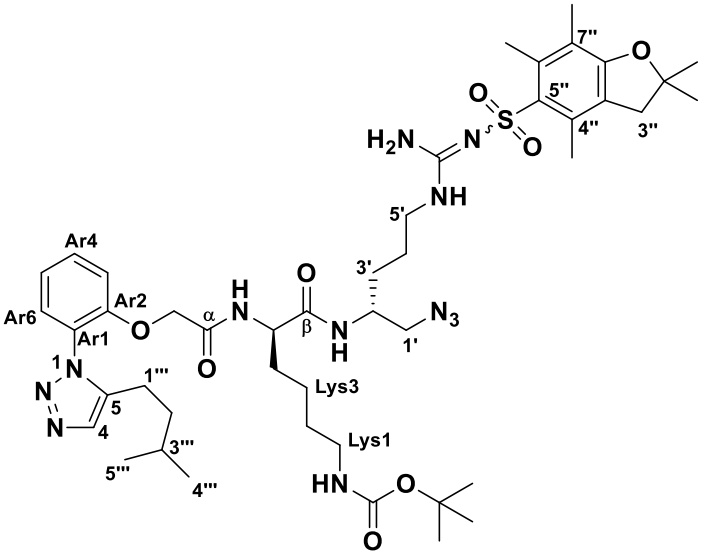
(*R*)-*N*-(1-Azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) guanidino)pentan-2-yl)-2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide (62)



Following **General Procedure III**, 2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetic acid **52** (0.14 g, 0.48 mmol), (*R*)-*N*-(amino((4-amino-5-azidopentyl)amino)methylene)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamide **56** (0.21 g, 0.48 mmol), EDCI.HCl (0.10 g, 0.53 mmol), HOBt (0.08 g, 0.53 mmol) and TEA (0.05 g, 0.48 mmol) were stirred in CH_2Cl_2 (3 mL) at rt for 16 h to give the acetamide **62** (0.22 g, 65%) as an off-white solid. M.P: 228 - 230 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (s, 1H, H4), 7.60-7.55 (m, 1H, Ar4), 7.33 (dd, *J* = 7.7, 1.5 Hz, 1H, Ar6), 7.24-7.20 (m, 1H, Ar5), 7.13 (d, *J* = 8.2 Hz, 1H, Ar3), 6.60 (brs, 1H, CON$\underline{H}$), 6.36 (brs, 1H, $N^{5'}$-H (guanidine)), 6.28 (brs, 2H, NH_2 (guanidine)), 4.57 (ABq, *J* = 14.8 Hz, 2H, $OC\underline{H}_A\underline{H}_B$), 4.00-3.92 (m, 1H, H2'), 3.60-3.23 (m,

4H, H1'/H5'), 2.95 (s, 2H, H3"), 2.60 (s, 3H, C4"-C$\underline{H}_3$), 2.58-2.54 (m, 2H, H1'"), 2.53 (s, 3H, C6"-C$\underline{H}_3$), 2.08 (s, 3H, C7"-C$\underline{H}_3$), 1.92-1.82 (m, 2H, H3'), 1.56-1.52 (m, 1H, H3'"), 1.52-1.42 (m, 4H, H2'"/H4'), 1.45 (s, 6H, C2"-(CH_3)$_2$), 0.83 (d, J = 6.3 Hz, 6H, H5'"/H4'"); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4 (C=O), 158.5 (C7a"), 156.3 (C=N), 152.1 (Ar2), 140.7 (C5), 138.3 (C3a"), 133.3 (C4), 132.35 (C4"), 132.30 (C6"), 131.7 (C5"), 128.0 (Ar4), 124.8 (Ar6), 124.4 (C7"), 122.4 (Ar1), 117.3 (Ar5), 113.6 (Ar3), 86.2 (C2"), 67.1 (O$\underline{C}H_AH_B$), 54.6 (C1'), 48.9 (C2'), 43.2 (C2'"), 40.7 (C5'), 37.0 (C3"), 29.1 (C3'), 28.6 (C2"-($\underline{C}H_3$)$_2$), 27.4 (C1'"), 25.3 (C4'), 22.1 (C4'"/C5'"), 22.0 (C3'"), 21.1 (C4"-C$\underline{H}_3$), 19.2 (C6"-C$\underline{H}_3$), 12.4 (C7"-C$\underline{H}_3$); IR (neat) $\bar{\nu}_{max}$ 3448, 3419, 3153, 3076, 2957, 2869, 2101, 1680, 1619, 1550, 1508, 1456, 1407, 1385, 1369, 1288, 1256, 1163, 1153, 1107, 1092, 1056, 1037, 994, 978, 852, 783, 734, 669, 642 cm^{-1}; MS (ESI +ve) *m/z* 731 ([M + Na]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{34}H_{48}N_{10}O_5SNa$ 731.3422, found 731.3423 ([M + Na]$^+$).

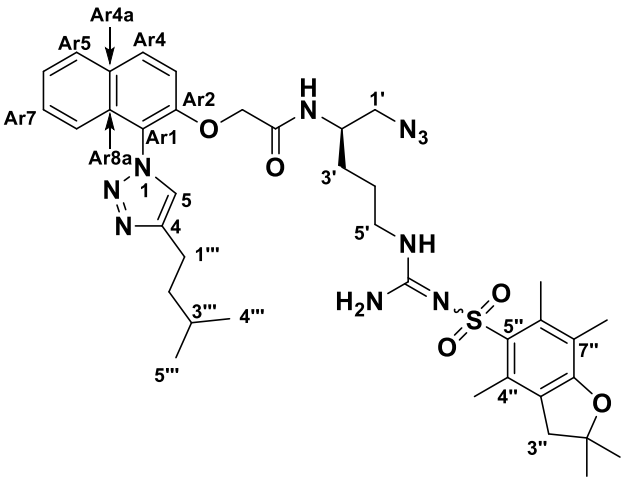
***Tert*-butyl ((*R*)-6-(((*R*)-1-azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-(2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamido)-6-oxohexyl)carbamate (63)**



Following **General Procedure III**, 2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetic acid **52** (0.03 g, 0.10 mmol), *tert*-butyl ((*R*)-5-amino-6-(((*R*)-1-azido-5-(2-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-6-oxohexyl)carbamate **57**

(0.07 g, 0.10 mmol), EDCI.HCl (0.02 g, 0.11 mmol), HOBt (0.02 g, 0.11 mmol) and TEA (0.01 g, 0.10 mmol) were stirred in CH_2Cl_2 (2 mL) at rt for 12 h to give the acetamide **63** (0.07 g, 74%) as an off-white solid. M.P: 242 - 244 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (s, 1H, H4), 7.54 (t, *J* = 8.1 Hz, 1H, Ar4), 7.52 (brs, 1H, βCONH), 7.34-7.14 (m, 3H, Ar6/Ar5/Ar3), 7.04-6.98 (brs, 1H, αCONH), 6.12-6.08 (m, 3H, $N^{5'}$-H/NH_2 (guanidine)), 5.20-5.14 (m, 1H, LysN^1-H), 4.65-4.53 (m, 2H, OCH_AH_B), 4.38-4.31 (m, 1H, Lys5), 3.99-3.96 (m, 1H, H2'), 3.30-3.24 (m, 2H, H1'), 3.10-3.01 (m, 4H, H5'/Lys1), 2.94 (s, 2H, H3''), 2.56 (s, 3H, C4''-CH_3), 2.49 (s, 3H, C6''-CH_3), 2.49-2.45 (m, 2H, H1'''), 2.07 (s, 3H, C7''-CH_3), 1.80-1.70 (m, 2H, Lys4), 1.54-1.40 (m, 9H, H3'/H4'/Lys2/H2'''/H3'''), 1.45 (s, 9H, $C(CH_3)_3$), 1.42 (s, 6H, C2''-$(CH_3)_2$), 1.30-1.26 (m, 2H, Lys3), 0.83 (d, *J* = 6.4 Hz, 3H, H4'''), 0.81 (d, *J* = 6.4 Hz, 3H, H5'''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.8 (βC=O), 168.4 (αC=O), 158.6 (C7a''), 156.2 (C=N), 152.1 ($COOC(CH_3)_3$), 140.2 (Ar2), 138.2 (C5), 133.1 (C4''), 132.1 (C6''), 132.0 (C3a''), 131.9 (C5''), 128.1 (C4), 128.0 (Ar4), 124.9 (C7''), 124.5 (Ar6), 122.4 (Ar1), 117.3 (Ar5), 113.8 (Ar3), 86.3 (C2''), 78.9 ($C(CH_3)_3$), 67.3 (OCH_AH_B), 54.6 (Lys5), 53.4 (C1'), 53.4 (C2'), 48.7 (C2'''), 43.2 (C5'), 40.6 (C3''), 40.1 (Lys1), 37.0 (Lys4), 31.5 (C3'), 29.2 (Lys2), 28.5 ($C(CH_3)_3$), 28.4 (C2''-$(CH_3)_2$), 28.2 (C1'''), 27.4 (C4'), 25.4 (C3'''), 22.8 (C4'''), 22.7 (C5'''), 22.1 (Lys3), 19.2 (C4''-CH_3), 17.9 (C6''-CH_3), 12.4 (C7''-CH_3); IR (neat) $\bar{\nu}_{max}$ 3319, 3073, 2953, 2933, 2869, 2101, 1662, 1602, 1546, 1508, 1459, 1420, 1390, 1366, 1251, 1195, 1165, 1109, 1057, 1024, 984, 913, 882, 833, 755, 665, 622, 580 cm^{-1}; MS (ESI +ve) *m/z* 959 ($[M + Na]^+$, 100%), 937 ($[M + H]^+$, 60%); HRMS (ESI +ve TOF) calcd for $C_{45}H_{69}N_{12}O_8S$ 937.5076, found 937.5077 ($[M + H]^+$).

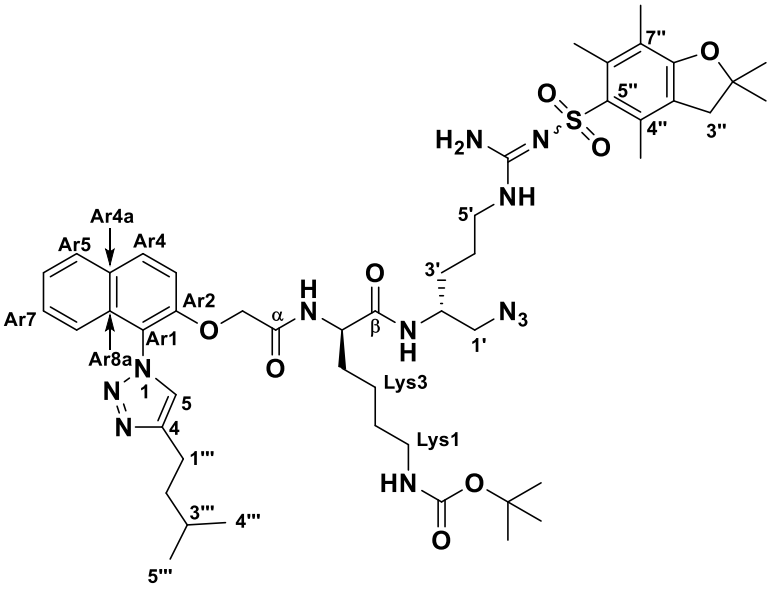
(*R*)-*N*-(1-Azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) guanidino)pentan-2-yl)-2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamide (64)



Following **General Procedure III,** 2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl) naphthalen-2-yl)oxy)acetic acid **53** (0.14 g, 0.41 mmol), (*R*)-*N*-(amino((4-amino-5-azidopentyl)amino)methylene)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamide **56** (0.18 g, 0.41 mmol), EDCI.HCl (0.09 g, 0.45 mmol), HOBt (0.07 g, 0.45 mmol) and TEA (0.04 g, 0.41 mmol) were stirred in CH_2Cl_2 (5 mL) at rt for 12 h to give the acetamide **64** (0.21 g, 67%) as an off-white solid. M.P: 238 - 240 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 9.2 Hz, 1H, Ar8), 7.92 (d, *J* = 8.2 Hz, 1H, Ar5), 7.66 (s, 1H, H5), 7.56-7.47 (m, 2H, Ar6/Ar7), 7.35 (d, *J* = 8.2 Hz, 1H, Ar4), 7.19 (d, *J* = 8.2 Hz, 1H, Ar3), 6.66 (brs, 1H, CON<u>H</u>), 6.54-6.58 (m, 3H, $N^{5'}$-H/ NH_2 (guanidine)), 4.69 (ABq, 14.8 Hz, 2H, OC<u>H</u>$_A$<u>H</u>$_B$), 3.99-3.94 (m, 1H, H2'), 3.55-3.20 (m, 4H, H1'/H5'), 2.93 (s, 2H, H3"), 2.91-2.88 (m, 2H, H1'"), 2.58 (s, 3H, C4"-C<u>H</u>$_3$), 2.52 (s, 3H, C6"-C<u>H</u>$_3$), 2.07 (s, 3H, C7"-C<u>H</u>$_3$), 1.78-1.76 (m, 2H, H3'), 1.74-1.58 (m, 3H, H2'"/H3'"), 1.52-1.46 (m, 2H, H4'), 1.44 (s, 6H, C2"-(C<u>H</u>$_3$)$_2$), 1.00 (d, *J* = 6.2 Hz, 6H, H4'"/H5'"); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5 (C=O), 158.5 (C7a"), 156.3 (Ar2), 149.9 (C=N), 149.1 (Ar8a), 138.3 (C3a"), 133.4 (C4"), 132.7 (C6"), 132.3 (C4), 130.3 (C5"), 129.2 (Ar4), 129.1 (Ar4a), 128.4 (C7"), 125.6 (Ar5), 124.9 (Ar7), 124.4 (Ar8), 120.6 (C5), 119.7 (Ar6), 117.3 (Ar3), 113.0 (Ar1), 86.2 (C2"), 67.6 (O<u>C</u>H$_A$H$_B$), 54.6 (C1'), 48.9 (C2'), 43.2 (C2'"), 40.7 (C5'), 38.4 (C3"), 29.1 (C3'), 28.5 (C2"-

($\underline{C}H_3)_2$), 27.8 (C1'''), 25.3 (C4'), 23.5 (C3'''), 22.4 (C4'''/C5'''), 19.2 (C4''-$\underline{C}H_3$), 17.9 (C6''-$\underline{C}H_3$), 12.4 (C7''-$\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 3435, 3418, 3332, 3141, 2954, 2869, 2100, 1677, 1627, 1600, 1548, 1483, 1452, 1407, 1383, 1369, 1278, 1254, 1150, 1107, 1091, 1044, 809, 782, 734, 664, 642, 619, cm^{-1}; MS (ESI +ve) *m/z* 781 ($[M + Na]^+$, 60%), 759 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{38}H_{50}N_{10}O_5SNa$ 781.3584, found 781.3620 ($[M + Na]^+$).

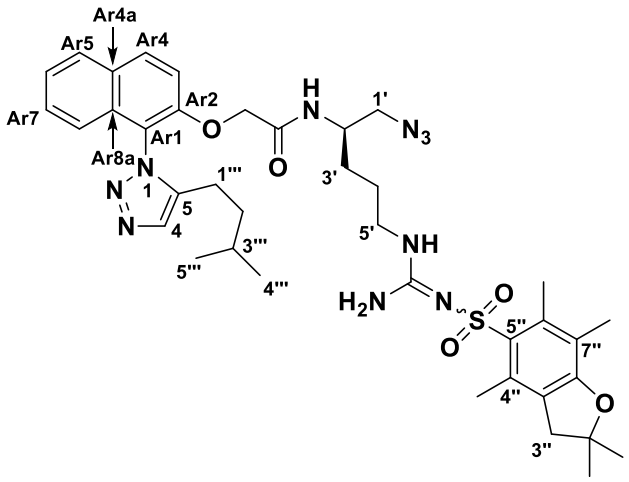
***Tert*-butyl ((*R*)-6-(((*R*)-1-azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-(2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido)-6-oxohexyl)carbamate (65)**



Following **General Procedure III**, 2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetic acid **53** (0.12 g, 0.35 mmol), *tert*-butyl ((*R*)-5-amino-6-(((*R*)-1-azido-5-(2-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-6-oxohexyl) carbamate **57** (0.24 g, 0.35 mmol), EDCI.HCl (0.08 g, 0.39 mmol), HOBt (0.06 g, 0.39 mmol) and TEA (0.03 g, 0.35 mmol) were stirred in CH_2Cl_2 (5 mL) at rt for 12 h to give the acetamide **65** (0.22 g, 64%) as an off-white solid. M.P: 236 – 238 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (d, *J* = 8.5 Hz, 1H, Ar8), 7.89 (d, *J* = 8.5 Hz, 1H, Ar5), 7.65 (s, 1H, H5), 7.54-7.47 (m, 2H, Ar4/βCON$\underline{H}$), 7.37 (d, *J* = 8.5 Hz, 1H, Ar7), 7.26-7.19 (m, 2H, Ar6/Ar3), 6.85 (brs, 1H,

αCONH), 6.36-6.08 (m, 3H, $N^{5'}$-H/NH_2 (guanidine)), 5.00 (brs, 1H, LysN^1-H), 4.69 (ABq, *J* = 16.5 Hz, 2H, OCH_AH_B), 4.42-4.36 (m, 1H, Lys5), 4.02-3.96 (m, 1H, H2'), 3.44-2.94 (m, 6H, H1'/H5'/Lys1), 2.88 (s, 2H, H3''), 2.88-2.84 (m, 2H, H1'''), 2.55 (s, 3H, C4''-CH_3), 2.48 (s, 3H, C6''-CH_3), 2.06 (s, 3H, C7''-CH_3), 2.00-1.86 (m, 4H, H4'/Lys4), 1.84-1.60 (m, 7H, H3'/Lys3/H2'''/H3'''), 1.44 (s, 6H, C2''(CH_3)$_2$), 1.39 (s, 9H, C(CH_3)$_3$), 1.32-1.22 (m, 2H, Lys2), 0.9 (d, *J* = 5.0 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.9 (βC=O), 168.1 (αC=O), 158.7 (C7a''), 156.4 (C=N), 150.1 (Ar2), 149.1 (COOC(CH_3)$_3$), 138.4 (Ar8a), 133.4 (C4), 132.6 (C4''), 132.46 (C6''), 132.44 (C3a''), 130.6 (C5''), 129.4 (Ar4), 129.1 (Ar4a), 128.48 (C7''), 128.47 (Ar5), 125.7 (Ar7), 124.7 (Ar8), 121.1 (C5), 120.3 (Ar6), 117.5 (Ar3), 113.8 (Ar1), 86.5 (C2''), 79.2 (C(CH_3)$_3$), 68.0 (OCH_AH_B), 54.8 (Lys5), 53.6 (C1'), 43.4 (C2'), 40.8 (C2'''), 40.2 (C5'), 38.6 (C3''), 38.5 (Lys1), 31.79 (Lys4), 31.74 (C3'), 29.4 (Lys2), 28.7 (C2''-(CH_3)$_2$), 28.6 ((CH_3)$_3$), 28.0 (C1'''), 25.5 (C4'), 23.8 (C3'''), 22.8 (C4'''/C5'''), 22.6 (Lys3), 19.4 (C4''-CH_3), 18.1 (C6''-CH_3), 12.6 (C7''-CH_3); IR (neat) $\bar{\nu}_{max}$ 3405, 3317, 3415, 3057, 2953, 2868, 2100, 1664, 1631, 1600, 1546, 1514, 1484, 1452, 1406, 1390, 1366, 1265, 1247, 1165, 1106, 1090, 1044, 994, 970, 852, 781, 733, 661, 641 cm^{-1}; MS (ESI +ve) *m/z* 987 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{49}H_{71}N_{12}O_8S$ 987.5239, found 987.5272 ([M + H]$^+$).

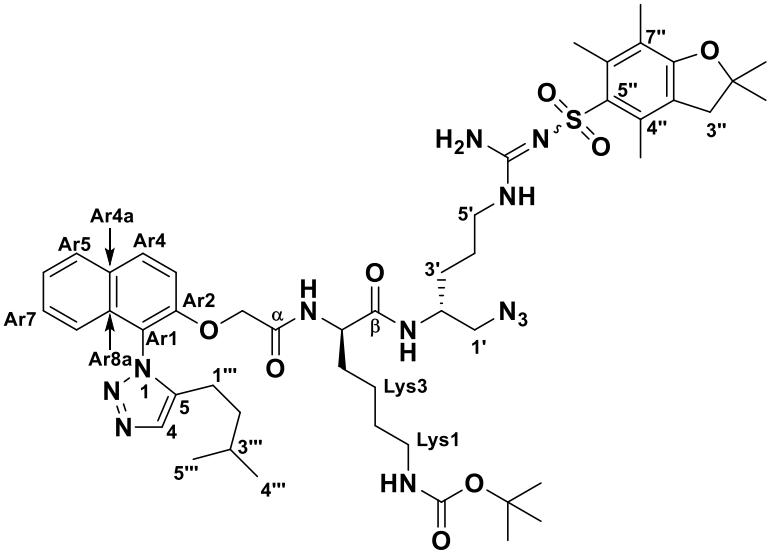
***N*-((*R*)-1-Azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl) guanidino)pentan-2-yl)-2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamide (66)**



Following **General Procedure III**, 2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetic acid **54** (0.15 g, 0.44 mmol), (*R*)-*N*-(amino((4-amino-5-azido pentyl)amino)methylene)-2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonamide **56** (0.19 g, 0.44 mmol), EDCI.HCl (0.09 g, 0.48 mmol), HOBt (0.07 g, 0.48 mmol) and TEA (0.04 g, 0.44 mmol) were stirred in CH_2Cl_2 (5 mL) at rt for 16 h to give the acetamide **66** (0.22 g, 66%) as an off-white solid. M.P: 248 - 250 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 7.3 Hz, 1H, Ar8), 7.94-7.92 (m, 1H, Ar5), 7.78 (s, 1H, H4), 7.55-7.49 (m, 2H, Ar6/Ar7), 7.41 (d, *J* = 7.4 Hz, 1H, Ar4), 7.04-7.02 (m, 1H, Ar3), 6.48 (brs, 1H, CON<u>H</u>), 6.35 (brs, 2H, NH_2 (guanidine)), 6.29 (brs, 1H, $N^{5'}$-H (guanidine)), 4.65 (ABq, *J* = 11.8 Hz, 2H, OC<u>H</u>$_A$<u>H</u>$_B$), 3.96-3.93 (m, 1H, H2'), 3.44-3.21 (m, 2H, H1'), 3.20-3.10 (m, 2H, H5'), 2.94 (s, 2H, H3''), 2.61 (s, 3H, C4''-C<u>H</u>$_3$), 2.54 (s, 3H, C6''-C<u>H</u>$_3$), 2.50-2.37 (m, 2H, H1'''), 2.08 (s, 3H, C7''-C<u>H</u>$_3$), 1.58-1.34 (m, 7H, H3'/H4'/H2'''/H3'''), 1.46 (s, 6H, C2''-(C<u>H</u>$_3$)$_2$), 0.73 (d, *J* = 5.0 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, $CDCl_3$, major rotamer) δ 167.9 (C=O), 158.5 (C7a''), 156.4 (Ar2), 150.5 (C=N), 150.4 (C5), 141.8 (Ar8a), 138.3 (C4''), 133.3 (C6''), 133.1 (C4), 132.3 (C3a''), 131.9 (C5''), 130.5 (Ar4), 129.3 (Ar4a), 129.1 (C7''), 128.5 (Ar5), 125.6 (Ar7), 124.4 (Ar8), 120.7 (Ar6), 118.3 (Ar3), 113.2 (Ar1), 86.2 (C2''), 67.3 (<u>C</u>H$_A$H$_B$), 54.4 (C1'), 48.7 (C2'), 43.2 (C2'''), 40.7 (C5'), 36.6 (C3''), 29.2 (C3'), 28.8 (C2''-

($\underline{C}H_3)_2$), 28.6 (C1'''), 27.2 (C4'), 25.4 (C3'''), 22.0 (C4'''/C5'''), 19.2 (C4''-$\underline{C}H_3$), 17.9 (C6''-$\underline{C}H_3$), 12.4 (C7''-$\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 3435, 3332, 3141, 3076, 2954, 2868, 2100, 1677, 1627, 1600, 1548, 1483, 1452, 1407, 1369, 1278, 1254, 1150, 1107, 1091, 1044, 994, 852, 809, 782, 749, 734, 664, 619 cm^{-1}; MS (ESI +ve) *m/z* 803 ([M + $HCOO^-$], 100%), 757 ([M - H], 90%); HRMS (ESI +ve TOF) calcd for $C_{38}H_{49}N_{10}O_5S$ 757.3608, found 757.3604 ([M - H]).

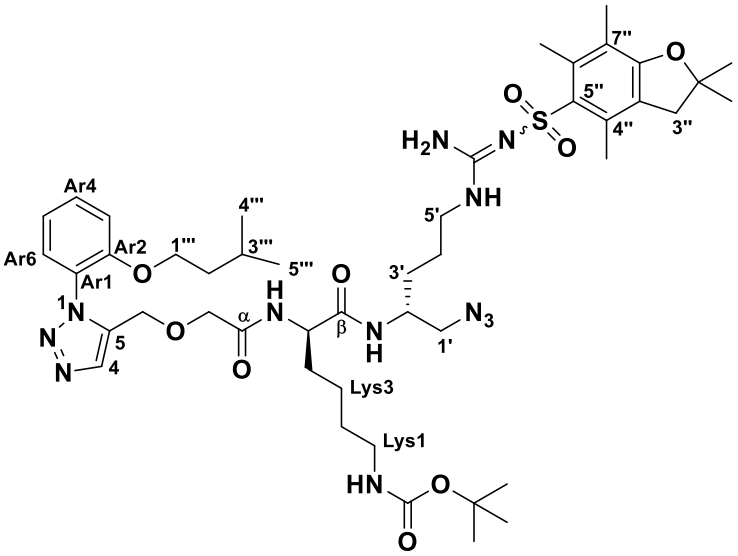
***Tert*-Butyl ((5*R*)-6-(((*R*)-1-azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-(2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido)-6-oxohexyl)carbamate (67)**



Following **General Procedure III**, 2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetic acid **54** (0.13 g, 0.38 mmol), *tert*-butyl ((*R*)-5-amino-6-(((*R*)-1-azido-5-(2-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-6-oxohexyl)carbamate **57** (0.26 g, 0.38 mmol), EDCI.HCl (0.08 g, 0.42 mmol), HOBt (0.06 g, 0.42 mmol) and TEA (0.04 g, 0.38 mmol) were stirred in CH_2Cl_2 (5 mL) at rt for 16 h to give the acetamide **67** (0.25 g, 67%) as a pale brown solid. M.P: 242 - 244 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14-8.09 (m, 1H, Ar8), 7.93 (d, *J* = 7.3 Hz, 1H, Ar5), 7.83 (s, 1H, H4), 7.66-7.36 (m, 3H, Ar4/βCON$\underline{H}$/Ar7), 7.08-6.95 (m, 2H, Ar6/Ar3), 6.54-6.52 (m, 1H, αCON$\underline{H}$), 6.34-6.24 (m, 3H, $N^{5'}$-H/NH_2 (guanidine)), 5.13 (brs, 1H, LysN^1-H), 4.64 (ABq, *J*

= 16.5 Hz, 2H, OC$\underline{H}_A\underline{H}_B$), 4.42-4.36 (m, 1H, Lys5), 4.02-3.96 (m, 1H, H2'), 3.30-2.94 (m, 6H, H1'/H5'/Lys1), 2.94 (s, 2H, H3''), 2.57 (s, 3H, C4''-C$\underline{H}_3$), 2.51 (s, 3H, C6''-C$\underline{H}_3$), 2.48-2.32 (m, 2H, H1'''), 2.08 (s, 3H, C7''-C$\underline{H}_3$), 1.82-1.76 (m, 2H, H4'), 1.58-1.16 (m, 11H, H3'/Lys2/Lys3/Lys4/H2'''/H3'''), 1.44 (s, 6H, C2''-(C$\underline{H}_3$)$_2$), 1.40 (s, 9H, C(C$\underline{H}_3$)$_3$), 0.74 (d, *J* = 5.5 Hz, 3H, H4'''), 0.71 (d, *J* = 6.0 Hz, 3H, H5'''); ^{13}C NMR (101 MHz, $CDCl_3$, major rotamer) δ 171.4 (βC=O), 167.6 (αC=O), 158.5 (C7a''), 156.2 (C=N), 150.6 (Ar2), 150.5 ($\underline{C}OOC(CH_3)_3$), 141.4 (C5), 138.2 (Ar8a), 133.2 (C4), 133.0 (C4''), 132.2 (C6''), 130.6 (C3a''), 129.3 (C5''), 129.0 (Ar4), 128.8 (Ar4a), 128.4 (C7''), 125.6 (Ar5), 124.5 (Ar7), 120.8 (Ar8), 118.2 (Ar6), 117.3 (Ar3), 113.5 (Ar1), 86.3 (C2''), 78.9 ($\underline{C}(CH_3)_3$), 67.7 (O$\underline{C}H_AH_B$), 54.5 (Lys5), 53.4 (C1'), 43.2 (C2'), 40.7 (C2'''), 40.2 (C5'), 36.7 (C3''), 31.8 (Lys1), 29.3 (Lys4), 29.1 (C3'), 28.5 (Lys2), 28.4 (C2''-($\underline{C}H_3$)$_2$), 28.4 (($\underline{C}H_3$)$_3$), 27.2 (C1'''), 25.4 (C4'), 22.9 (C3'''), 22.0 (C4'''/C5'''), 20.9 (Lys3), 19.2 (C4''-$\underline{C}H_3$), 17.9 (C6''-$\underline{C}H_3$), 12.4 (C7''-$\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 3406, 3317, 3144, 3057, 2953, 2868, 2100, 1664, 1631, 1600, 1545, 1514, 1484, 1452, 1406, 1390, 1366, 1265, 1165, 1151, 1090, 1044, 1024, 994, 913, 852, 807, 781, 733, 660, 641, 566 cm^{-1}; MS (ESI +ve) *m/z* 988 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{49}H_{72}N_{12}O_8S$ 988.5317, found 988.5337 ([M+H]$^+$).

***Tert*-Butyl ((*R*)-6-(((*R*)-1-azido-5-(2-((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)guanidino)pentan-2-yl)amino)-5-(2-((1-(2-(isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)acetamido)-6-oxohexyl)carbamate (68)**

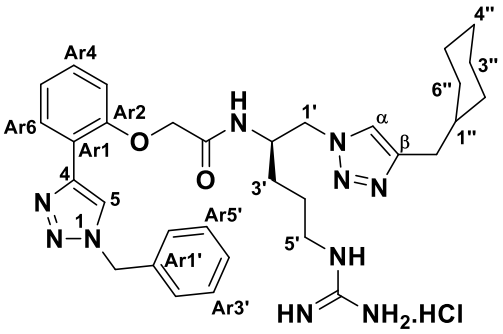


Following **General Procedure III**, 2-((1-(2-(isopentyloxy) phenyl)-1*H*-1,2,3-triazol-5-yl) methoxy)acetic acid **55** (0.12 g, 0.37 mmol), *tert*-butyl ((*R*)-5-amino-6-(((*R*)-1-azido-5-(2-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl) sulfonyl)guanidino)pentan-2-yl)amino)-6-oxohexyl) carbamate **57** (0.25 g, 0.37 mmol), EDCI.HCl (0.08 g, 0.41 mmol), HOBt (0.06 g, 0.41 mmol) and TEA (0.04 g, 0.37 mmol) were stirred in CH_2Cl_2 (3 mL) at rt for 16 h to give the acetamide **68** (0.25 g, 70%) as a pale brown solid. M.P: 220 - 222 °C. TLC (MeOH/ CH_2Cl_2 - 10:90): R_f = 0.6; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 1H, H4), 7.49 (ddd, *J* = 8.2, 1.6 Hz, 1H, Ar4), 7.40 (dd, *J* = 8.0, 1.6 Hz, 1H, Ar6), 7.11-7.0 (m, 3H, Ar5/Ar3/βCONH), 6.97 (brs, 1H, αCONH), 6.34-6.24 (m, 3H, $N^{5'}$-H/NH_2 (guanidine)), 4.75 (t, *J* = 5.8 Hz, 1H, LysN^1-H), 4.63-4.55 (m, 2H, C5-CH_2), 4.35 (t, *J* = 6.5 Hz, 1H, Lys5), 4.01-3.74 (m, 3H, H2'/H1'''), 3.89-3.74 (m, 2H, OCH_AH_B), 3.38-3.28 (m, 2H, H1'), 3.44-3.40 (m, 2H, H5'), 3.07-3.00 (m, 2H, Lys1), 2.94 (s, 2H, H3''), 2.56 (s, 3H, C4''-CH_3), 2.50 (s, 3H, C6''-CH_3), 2.08 (s. 3H, C7''-CH_3), 1.85-1.80 (m, 2H, Lys4), 1.64-1.45 (m, 9H, H2'''/H3'''/Lys2/H3'/H4'), 1.45 (s, 6H, C2''(CH_3)$_2$), 1.41 (s, 9H, C(CH_3)$_3$), 1.36-1.20 (m, 2H, Lys3), 0.82 (d, *J* = 6.3 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.7 (βC=O), 169.1 (αC=O), 158.7 (C7a''), 156.2 (C=N), 153.1 (COO$(CH_3)_3$),

138.3 (Ar2), 135.0 (C5), 133.1 (C4"), 132.9 (C6"), 132.2 (C3a"), 131.8 (C4), 128.3 (C5"), 124.9 (C7"), 124.8 (Ar4), 124.6 (Ar6), 121.1 (Ar1), 117.4 (Ar5), 113.3 (Ar3), 86.4 (C2"), 79.2 ($\underline{C}(CH_3)_3$), 69.1 ($O\underline{C}H_AH_B$), 67.7 (C1'''), 62.1 (C5-$\underline{C}H_2$), 54.6 (Lys5), 52.7 (C1'), 43.2 (C2'), 40.7 (C5'), 39.9 (C3"), 37.5 (Lys1), 32.0 (C2'''), 29.7 (Lys4), 29.3 (Lys2), 29.2 (C3'), 28.6 (C2"-($\underline{C}H_3)_2$), 28.4 (($\underline{C}H_3)_3$), 25.5 (C4'), 24.9 (C3'''), 22.5 (C4'''/C5'''), 22.4 (Lys3), 19.2 (C4"-$\underline{C}H_3$), 17.9 (C6"-$\underline{C}H_3$), 12.4 (C7"-$\underline{C}H_3$); IR (neat) $\bar{\nu}_{max}$ 3325, 3147, 3076, 2963, 2930, 2869, 2100, 1661, 1620, 1549, 1510, 1459, 1404, 1390, 1367, 1286, 1250, 1166, 1107, 1050, 1036, 996, 978, 852, 808, 783, 757, 734, 663, 642, 569 cm^{-1}; MS (ESI +ve) *m/z* 989 ($[M + Na]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{46}H_{70}N_{12}O_9SNa$ 989.5007, found 989.5029 ($[M + Na]^+$).

6.3.4 – Synthesis of the derivatives 21 – 49

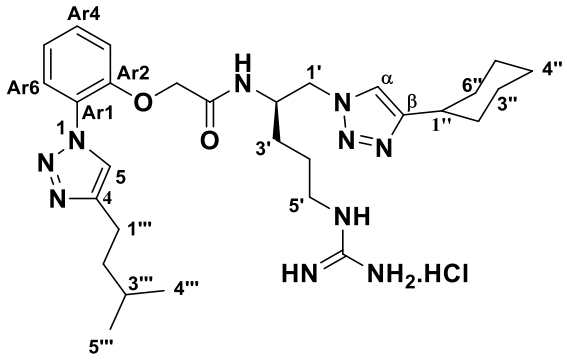
(*R*)-2-(2-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)phenoxy)-N-(1-(4-(cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)acetamide hydrochloride (21)



Following **General Procedure IV**, azide **58** (0.08 g, 0.11 mmol), 3-cyclohexyl-1-propyne (0.04 g, 0.33 mmol), $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.009 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **133** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 4:96). Following **General Procedure VII**, the intermediate **133** (0.07 g, 0.08 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.6 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **21** (0.03 g, 43% over two steps) as a pale brown solid that rapidly transitioned to a sticky

gum. $[\alpha]_D^{23}$ +63.1 (*c* 0.0053, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.59 (s, 1H, H5), 7.98 (s, 1H, Hα), 7.80 (apparent t, *J* = 7.5 Hz, 1H, Ar4), 7.44-7.30 (m, 6H, Ar6/Ar2'/Ar3'/Ar4'/Ar5'/Ar6'), 7.09 (apparent t, *J* = 7.5 Hz, 1H, Ar5), 6.99 (d, *J* = 7.5 Hz, 1H, Ar3), 4.88-4.72 (m, 3H, OC$\underline{H}_A\underline{H}_B$/H1'), 4.66-4.52 (m, 3H, H1'/C$\underline{H}_2$Ph), 4.48-4.42 (m, 1H, H2'), 3.22-3.12 (m, 2H, H5'), 2.36-2.24 (m, 3H, βC-C$\underline{H}_2$/H1''), 1.84-1.40 (m, 9H, H3'/H4'/H2''/H3''/H4''/H5''/H6''), 1.16-1.02 (m, 3H, H2''/H4''/H6''), 0.88-0.78 (m, 2H, H3''/H5''); ^{13}C NMR (101 MHz, CD_3OD) δ 171.7 (C=O), 158.7 (C=N), 155.9 (Ar2), 145.8 (C4), 145.6 (Ar1'), 136.8 (Ar6), 131.3 (Cβ), 131.2 (C5), 130.2 (Ar4), 129.7 (Ar3'), 129.6 (Ar5'), 129.5 (Ar2'), 129.3 (Ar6'), 127.4 (Ar4'), 125.4 (Cα), 123.4 (Ar5), 120.2 (Ar1), 114.4 (Ar3), 68.8 (O$\underline{C}H_AH_B$), 56.2 (C1'), 55.4 ($\underline{C}H_2$Ph), 50.7 (C2'), 42.0 (C5'), 39.0 (C1''), 33.8 (βC-$\underline{C}H_2$), 32.7 (C2''/C6''), 29.9 (C3'), 27.3 (C4''), 27.1 (C3''/C5''), 26.4 (C4'); IR (neat) $\bar{\nu}_{max}$ 3343, 3272, 3184, 3072, 2926, 2853, 2475, 1900, 1667, 1585, 1549, 1489, 1449, 1351, 1289, 1224, 1179, 1167, 1127, 1079, 1049, 978, 936, 858, 759, 723, 696, 672, 648, 584 cm^{-1}; MS (ESI +ve) *m/z* 599 ([M – HCl + H]$^+$, 100%), 300 ([M – HCl + H]$^{2+}$, 70%); HRMS (ESI +ve TOF) calcd for $C_{32}H_{43}N_{10}O_2$ 599.3565, found 599.3562 ([M – HCl + H]$^+$).

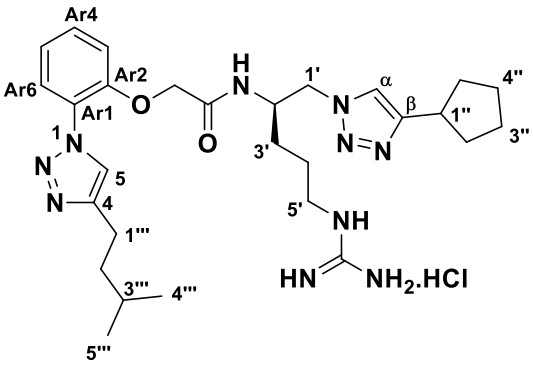
(*R*)-*N*-(1-(4-Cyclohexyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide hydrochloride (22)



Following **General Procedure IV**, azide **60** (0.07 g, 0.09 mmol), cyclohexylacetylene (0.03 g, 0.27 mmol), $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **134** as an off-white solid after flash

chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 3:97). Following **General Procedure VII**, the intermediate **134** (0.06 g, 0.07 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.46 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **22** (0.03 g, 55% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +68.1 (*c* 0.0047, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (s, 1H, H5), 8.36 (s, 1H, Hα), 7.65 (apparent t, *J* = 7.5 Hz, 1H, Ar4), 7.55 (d, *J* = 7.3 Hz, 1H, Ar6), 7.28-7.18 (m, 2H, Ar5/Ar3), 4.84-4.78 (m, 1H, H1'), 4.72-4.58 (m, 3H, OC$\underline{H}_A\underline{H}_B$/H1'), 4.50-4.40 (m, 1H, H2'), 3.30-3.20 (m, 2H, H5'), 2.90-2.80 (m, 2H, H1'''), 2.04-1.94 (m, 2H, H3'), 1.86-1.50 (m, 11H, H4'/H2'''/H3'''/H1''/H2''/H3''/H4''/H5''/H6''), 1.48-1.20 (m, 5H, H2''/H3''/H4''/H5''/H6''), 0.99 (d, *J* = 5.0 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 170.9 (C=O), 158.7 (C=N), 152.1 (Ar2), 147.9 (Cβ), 132.8 (C4), 127.7 (Ar4), 127.3 (Ar6), 125.9 (C5), 125.3 (Cα), 123.8 (Ar5), 122.3 (Ar3), 115.7 (Ar1), 69.1 (C1'), 67.0 (O$\underline{C}H_AH_B$), 57.2 (C2'), 50.7 (C2'''), 42.1 (C5'), 39.6 (C1''), 33.3 (C2''), 33.2 (C6''), 29.8 (C3'), 29.0 (C1'''), 26.8 (C4''), 26.7 (C3''), 26.5 (C5''), 24.1 (C3'''), 23.6 (C4'), 22.9 (C4'''/C5'''); IR (neat) $\bar{\nu}_{max}$ 3339, 3276, 3180, 2931, 2857, 2481, 1906, 1670, 1603, 1549, 1507, 1465, 1451, 1386, 1368, 1289, 1230, 1168, 1130, 1048, 981, 937, 855, 821, 800, 761, 701, 669 cm^{-1}; MS (ESI +ve) *m/z* 565 ([M – HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{29}H_{45}N_{10}O_2$ 565.3727, found 565.3731 ([M – HCl + H]$^+$).

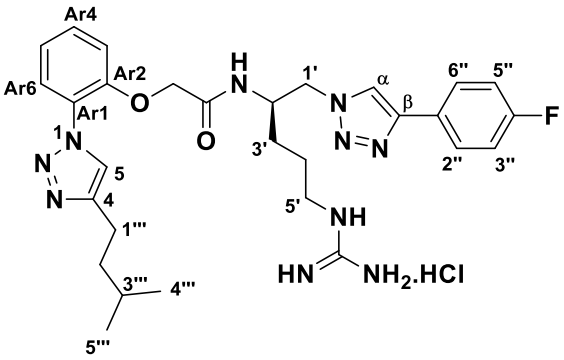
(*R*)-*N*-(1-(4-Cyclopentyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide hydrochloride (23)



Following **General Procedure IV**, azide **60** (0.07 g, 0.09 mmol), cyclopentylacetylene (0.03 g, 0.29 mmol), $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **135** as an off-white solid after flash chromatography over SiO_2 gel (MeOH/CH_2Cl_2 – 0:100 → 3:97). Following **General Procedure VII**, the intermediate **135** (0.06 g, 0.07 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.4 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **23** (0.025 g, 57% over two steps) as a brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +58.0 (*c* 0.0052, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.58 (s, 1H, H5), 8.45 (brs, 1H, Hα), 7.68-7.58 (m, 1H, Ar6), 7.56-7.46 (m, 1H, Ar4), 7.22-7.06 (m, 2H, Ar5/Ar3), 4.70-4.56 (m, 2H, OC$\underline{H}_A\underline{H}_B$), 4.50-4.40 (m, 1H, H1'), 4.38-4.30 (m, 1H, H1'), 4.20-4.12 (m, 1H, H2'), 3.14-3.02 (m, 3H, H5'/H1''), 2.76-2.62 (m, 2H, H1'''), 1.96-1.84 (m, 2H, H3'), 1.68-1.38 (m, 13H, H4'/H2'''/H3'''/H2''/H3''/H4''/H5''), 0.90 (d, *J* = 5.6 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 167.2 (C=O), 156.7 (C=N), 150.6 (Ar2), 149.6 (Cβ), 146.7 (C4), 129.9 (Ar4), 125.9 (Ar6), 125.1 (Cα), 123.5 (C5), 121.6 (Ar5), 121.5 (Ar3), 113.9 (Ar1), 69.7 (C1'), 58.4 (O$\underline{C}H_AH_B$), 50.7 (C2'), 42.3 (C2'''), 39.2 (C5'), 36.1 (C1''), 33.9 (C3'), 29.8 (C1'''), 28.9 (C2''/C5''), 26.7 (C3''/C4''), 26.2 (C3'''), 24.0 (C4'), 22.9 (C4'''/C5'''); IR (neat) $\bar{\nu}_{max}$ 3344, 3276, 3184, 2957, 2871, 2478, 2110, 1895, 1722, 1668, 1603, 1551, 1505, 1469, 1453, 1385, 1368, 1288, 1229, 1168, 1131, 1080, 1050, 993, 939, 854, 801, 760, 653,

586 cm^{-1}; MS (ESI +ve) *m/z* 551 ($[M - HCl + H]^+$, 100%), 276 ($[M - HCl + H]^{2+}$, 80%); HRMS (ESI +ve TOF) calcd for $C_{28}H_{43}N_{10}O_2$ 551.3565, found 551.3572 ($[M - HCl + H]^+$).

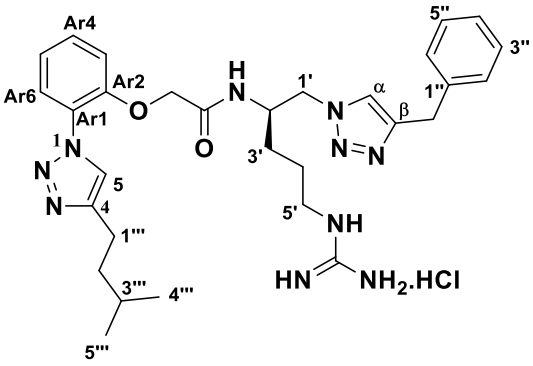
(*R*)-*N*-(1-(4-(4-Fluorophenyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide hydrochloride (24)



Following **General Procedure IV**, azide **60** (0.06 g, 0.09 mmol), 1-ethynyl-4-fluoro benzene (0.03 g, 0.27 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **136** as an off-white solid after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 3:97). Following **General Procedure VII**, the intermediate **136** (0.06 g, 0.07 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.4 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **24** (0.03 g, 54% over two steps) as an off-white solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +74.2 (*c* 0.0051, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H, H5), 8.39 (brs, 2H, N^5-H/C=N<u>H</u>), 7.86-7.78 (m, 3H, N<u>H</u>$_2$.<u>H</u>Cl), 7.59 (d, *J* = 7.5 Hz, 1H, Ar6), 7.50-7.38 (m, 1H, CON<u>H</u>), 7.32-7.22 (m, 4H, Hα/Ar4/H2"/H6"), 7.08 (t, *J* = 7.5 Hz, 1H, Ar3), 7.02 (d, $J_{H\text{-}F}$ = 8.5 Hz, 2H, H3"/H5"), 7.02-6.90 (m, 1H, Ar5), 4.65 (ABq, *J* = 15.8 Hz, 2H, OC<u>H</u>$_A$<u>H</u>$_B$), 4.54-4.40 (m, 2H, H1'), 4.28-4.18 (m, 1H, H2'), 3.12-3.02 (m, 2H, H5'), 2.64 (t, *J* = 7.5 Hz, 2H, H1'''), 1.62-1.40 (m, 7H, H3'/H4'/H2'''/H3'''), 0.88 (d, *J* = 6.5 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, DMSO-d_6) δ 167.5 (C=O), 161.7 (d, $J_{C\text{-}F}$ = 196.4 Hz, C4"), 156.9 (C=N), 149.7 (Ar2), 146.9 (Cβ), 145.3 (C4), 130.0 (Ar4), 127.3 (d, $J_{C\text{-}F}$ = 3.0 Hz, C1"), 127.2 (Ar6), 127.1 (Cα), 126.1 (C5), 125.3

(Ar5), 123.7 (Ar3), 121.8 (d, $J_{C\text{-}F}$ = 22.3 Hz, C2"/C6"), 115.8 (d, $J_{C\text{-}F}$ = 16.9 Hz, C3"/C5"), 114.0 (Ar1), 67.3 (C1'), 64.9 (O<u>C</u>H_AH_B), 52.7 (C2'), 48.7 (C2'"), 40.3 (C5'), 28.3 (C3'), 27.1 (C1'"), 25.0 (C3'"), 22.9 (C4'), 22.3 (C4'"/C5'"); IR (neat) $\bar{\nu}_{max}$ 3342, 3273, 3182, 2957, 2871, 2489, 1895, 1667, 1559, 1501, 1469, 1414, 1387, 1367, 1286, 1230, 1191, 1166, 1132, 1081, 1049, 993, 975, 941, 843, 816, 759, 666, 654, 600, 585 cm^{-1}; MS (ESI +ve) *m/z* 577 ([M – HCl + H]$^+$, 100%), 289 ([M – HCl + H]$^{2+}$, 20%); HRMS (ESI +ve TOF) calcd for $C_{29}H_{37}N_{10}O_2ClF$ 611.2768, found 611.2771 ([M + H]$^+$).

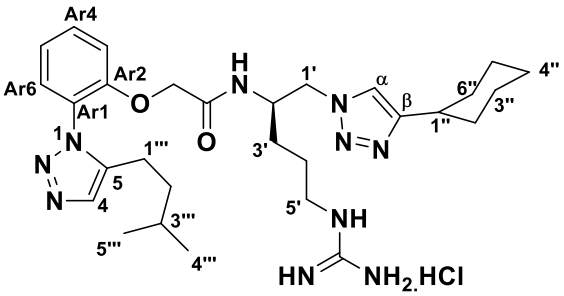
(*R*)-*N*-(1-(4-Benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide hydrochloride (25)



Following **General Procedure IV**, azide **60** (0.07 g, 0.09 mmol), 3-phenyl-1-propyne (0.03 g, 0.27 mmol), $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **137** as an off-white gum after flash chromatography over SiO_2 gel (MeOH/CH_2Cl_2 – 0:100 → 3:97). Following **General Procedure VII**, the intermediate **137** (0.06 g, 0.07 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.4 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **25** (0.029 g, 53% over two steps) as a brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +68.1 (*c* 0.0042, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.76 (brs, 1H, H5), 8.38 (brs, 1H, Hα), 7.68 (d, *J* = 7.5 Hz, 1H, Ar6), 7.55 (t, *J* = 8.0 Hz, 1H, Ar4), 7.32-7.20 (m, 6H, Ar5/H2"/H3"/H4"/H5"/H6"), 7.15 (d, *J* = 8.0 Hz, 1H, Ar3), 4.82-4.76 (m, 1H, OC<u>H</u>$_A$H$_B$), 4.68-4.56 (m, 3H, OCH$_A$<u>H</u>$_B$/H1'), 4.48-4.40 (m, 1H, H2'), 4.15 (s, 2H, C<u>H</u>$_2$Ph),

3.28-3.16 (m, 2H, H5'), 2.92-2.84 (m, 2H, H1'''), 1.84-1.50 (m, 7H, H3'/H4'/H2'''/H3'''), 0.99 (d, *J* = 4.0 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 170.4 (C=O), 158.6 (C=N), 151.8 (Ar2), 137.6 (Cβ), 133.1 (C4), 131.1 (C1''), 130.8 (Ar4), 130.0 (C2''/C6''), 129.8 (Ar6), 129.7 (C3''/C5''), 128.34 (C5), 128.33 (C4''), 127.2 (Cα), 123.64 (Ar5), 123.63 (Ar3), 115.4 (Ar1), 68.7 (C1'), 66.8 (O<u>C</u>H_AH_B), 56.8 (C2'), 50.5 (C2'''), 41.9 (C5'), 38.8 (C3'), 29.5 (<u>C</u>H_2Ph), 28.8 (C1'''), 26.2 (C3'''), 23.4 (C4'), 22.6 (C4'''/C5'''); IR (neat) $\bar{\nu}_{max}$ 3338, 3274, 3182, 3073, 2957, 2934, 2870, 2470, 1902, 1669, 1603, 1549, 1507, 1465, 1386, 1368, 1288, 1230, 1167, 1132, 1049, 983, 936, 855, 799, 760, 721, 697, 669, 644 cm^{-1}; MS (ESI +ve) *m/z* 573 ([M – HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{30}H_{41}N_{10}O_2$ 573.3414, found 573.3434 ([M – HCl + H]$^+$).

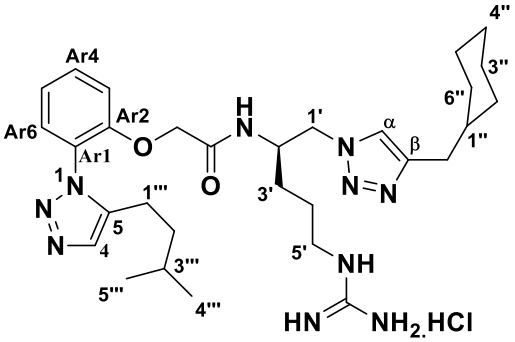
(*R*)-*N*-(1-(4-Cyclohexyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide hydrochloride (26)



Following **General Procedure IV**, azide **62** (0.07 g, 0.09 mmol), cyclohexylacetylene (0.03 g, 0.27 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **138** as a pale brown solid after flash chromatography over SiO_2 gel (MeOH/CH_2Cl_2 – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **138** (0.06 g, 0.07 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.46 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **26** (0.03 g, 56% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +59.7 (*c* 0.0058, MeOH). ^{1}H NMR (400

MHz, CD_3OD) δ 7.98 (d, *J* = 8.4 Hz, 1H, Ar4), 7.75 (s, 1H, H4), 7.74 (s, 1H, Hα), 7.63-7.57 (m, 1H, Ar6), 7.48 (dd, *J* = 7.7, 1.6 Hz, 1H, Ar5), 7.09 (d, *J* = 8.4 Hz, 1H, Ar3), 4.59 (ABq, *J* = 15.1 Hz, 2H, $OC\underline{H}_A\underline{H}_B$), 4.51-4.46 (m, 1H, H1'), 4.40-4.33 (m, 1H, H1'), 4.26-4.16 (m, 1H, H2'), 3.18-3.08 (m, 2H, H1'''), 2.72-2.62 (m, 2H, H5'), 2.00-1.88 (m, 2H, H3'), 1.84-1.68 (m, 3H, H1''/H2'''), 1.62-1.18 (m, 13H, H3'''/H2''/H3''/ H4''/H5''/H6''/H4'), 0.82 (d, *J* = 6.4 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 167.4 (C=O), 157.3 (C=N), 152.8 (Ar2), 152.0 (Cβ), 140.4 (C5), 133.4 (C4), 132.0 (Ar4), 131.6 (Ar6), 128.9 (Ar1), 125.0 (Cα), 121.8 (Ar5), 114.0 (Ar3), 67.1 ($O\underline{C}H_AH_B$), 53.0 (C1'), 49.0 (C2'), 36.8 (C5'), 34.8 (C2'''), 32.89 (C1''), 32.85 (C2''/C6''), 28.9 (C3'), 27.3 (C3'''), 26.0 (C1'''), 25.9 (C4''), 25.3 (C3''/C5''), 22.4 (C4'), 20.7 (C4'''/C5'''); IR (neat) $\bar{\nu}_{max}$ 3339, 3276, 3185, 2932, 2858, 2476, 1907, 1670, 1603, 1549, 1507, 1465, 1451, 1386, 1368, 1289, 1230, 1169, 1131, 1048, 983, 954, 938, 855, 822, 800, 762, 700, 669, 643 cm^{-1}; MS (ESI +ve) *m/z* 565 ($[M - HCl + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{29}H_{45}N_{10}O_2$ 565.3727, found 565.3734 ($[M + H]^+$).

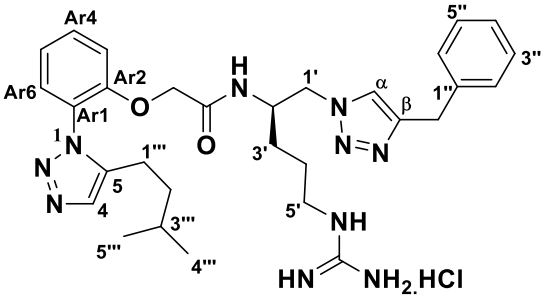
(*R*)-*N*-(1-(4-(Cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide hydrochloride (27)



Following **General Procedure IV**, azide **62** (0.07 g, 0.09 mmol), 3-cyclohexyl-1-propyne (0.03 g, 0.27 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **139** as a white solid after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **139** (0.07 g, 0.08 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.6 mmol)

and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **27** (0.03 g, 54% over two steps) as a pale-yellow solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +62.1 (*c* 0.0051, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (brs, 1H, H4), 7.95 (brs, 1H, Hα), 7.63 (t, *J* = 7.4 Hz, 1H, Ar4), 7.47 (d, *J* = 7.4 Hz, 1H, Ar6), 7.30-7.22 (m, 2H, Ar5/Ar3), 4.79-4.69 (m, 1H, H1'), 4.63-4.49 (m, 3H, OC$\underline{H}_A\underline{H}_B$/H1'), 4.47-4.39 (m, 1H, H2'), 3.22-3.10 (m, 2H, H1'''), 2.77-2.61 (m, 4H, βC-C$\underline{H}_2$/H5'), 1.84-1.36 (m, 13H, H3'/H4'/H2'''/H3'''/H1''/H2''/H3''/H4''/H5''/H6''), 1.34-1.09 (m, 3H, H2''/H4''/H6''), 1.04-0.90 (m, 2H, H3''/H5''), 0.81 (d, *J* = 6.1 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 168.7 (C=O), 157.2 (C=N), 152.5 (Ar2), 145.6 (Cβ), 140.8 (C5), 140.1 (Ar4), 132.6 (C4), 131.7 (Ar6), 128.5 (Ar1), 123.8 (Cα), 122.2 (Ar5), 113.9 (Ar3), 67.1 (O$\underline{C}H_AH_B$), 56.1 (C1'), 49.1 (C2'), 40.5 (C5'), 37.3 (C2'''), 36.6 (C1''), 32.2 (βC-$\underline{C}H_2$), 30.6 (C2''/C6''), 28.2 (C3'), 27.2 (C3'''), 25.7 (C1'''), 25.6 (C4''), 24.9 (C3''/C5''), 21.1 (C4'), 20.9 (C4'''/C5'''); IR (neat) $\bar{\nu}_{max}$ 3345, 3275, 3186, 2953, 2927, 2854, 2482, 1909, 1670, 1603, 1550, 1507, 1465, 1450, 1386, 1368, 1288, 1229, 1168, 1130, 1084, 1049, 983, 958, 935, 854, 761, 669, 648, 585 cm^{-1}; MS (ESI +ve) *m/z* 579 ([M – HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{30}H_{47}N_{10}O_2$ 579.3877, found 579.3876 ([M – HCl + H]$^+$).

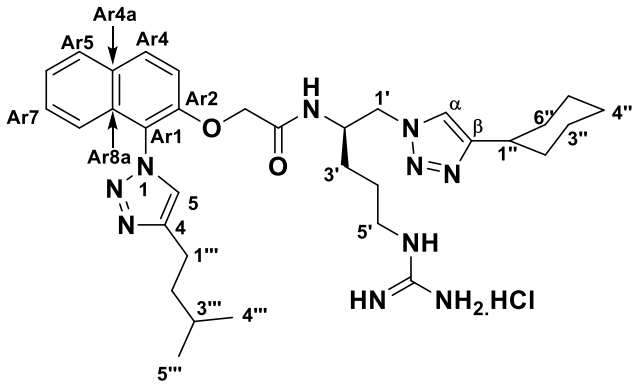
(*R*)-*N*-(1-(4-Benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamide hydrochloride (28)



Following **General Procedure IV**, azide **62** (0.07 g, 0.09 mmol), 3-phenyl-1-propyne (0.03 g, 0.27 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O

(0.5 mL) for 16 h to give the intermediate **140** as an off-white gum after flash chromatography over SiO_2 gel (MeOH/CH_2Cl_2 – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **140** (0.07 g, 0.08 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.6 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **28** (0.03 g, 55% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +73.6 (*c* 0.0046, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.22 (brs, 1H, H4), 8.11 (brs, 1H, Hα), 7.62 (t, *J* = 7.7 Hz, 1H, Ar4), 7.48 (d, *J* = 7.3 Hz, 1H, Ar6), 7.31-7.22 (m, 6H, Ar5/H2"/H3"/H4"/H5"/H6"), 7.17 (d, *J* = 7.7 Hz, 1H, Ar3), 4.80-4.70 (m, 1H, H1'), 4.52-4.44 (m, 3H, OC$\underline{H}_A\underline{H}_B$/H1'), 4.42-4.32 (m, 1H, H2'), 4.16 (s, 2H, C$\underline{H}_2$Ph), 3.24-3.12 (m, 2H, H1'"), 2.72-2.62 (m, 2H, H5'), 1.83-1.42 (m, 7H, H3'/H4'/H2'"/H3'"), 0.81 (d, *J* = 5.6 Hz, 6H, H4'"/H5'"); ^{13}C NMR (101 MHz, CD_3OD) δ 167.4 (C=O), 157.3 (C=N), 152.8 (Ar2), 146.1 (Cβ), 140.4 (C5), 139.9 (C1"), 132.0 (C4), 131.6 (Ar4), 128.9 (C2"/C6"), 128.8 (C3"/C5"), 128.7 (Ar6), 126.5 (C4"), 125.0 (Ar1), 123.6 (Cα), 121.9 (Ar5), 114.0 (Ar3), 67.0 (O$\underline{C}H_AH_B$), 55.3 (C1'), 52.9 (C2'), 40.5 (C5'), 36.7 (C2'"), 31.6 ($\underline{C}H_2$Ph), 28.9 (C3'), 27.3 (C3'"), 25.3 (C1'"), 22.4 (C4'), 20.7 (C4'"/C5'"); IR (neat) $\bar{\nu}_{max}$ 3341, 3276, 3181, 3088, 3075, 2956, 2934, 2870, 2470, 1914, 1669, 1603, 1549, 1507, 1465, 1385, 1368, 1288, 1229, 1168, 1131, 1049, 982, 936, 855, 799, 759, 721, 698, 669, 645 cm^{-1}; MS (ESI +ve) *m/z* 573 ([M – HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{30}H_{41}N_{10}O_2$ 573.3414, found 573.3420 ([M – HCl + H]$^+$).

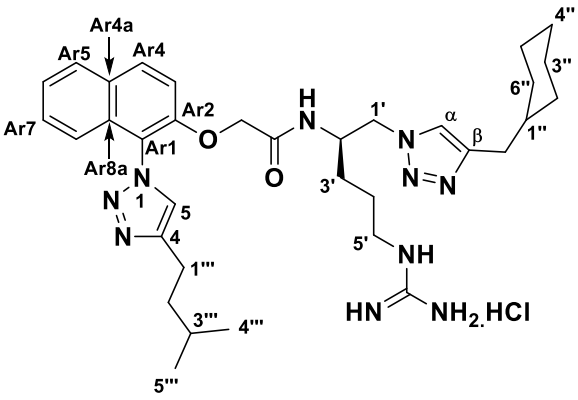
(*R*)-*N*-(1-(4-Cyclohexyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamide hydrochloride (29)



Following **General Procedure IV**, azide **64** (0.07 g, 0.09 mmol), cyclohexyl acetylene (0.03 g, 0.27 mmol), $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.01 mmol) and sodium ascorbate (0.008 g, 0.03 mmol) were stirred in *t*-BuOH (2.0 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **141** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 6:94). Following **General Procedure VII**, the intermediate **141** (0.06 g, 0.06 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.26 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **29** (0.03 g, 72% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +58.1 (*c* 0.0061, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.36 (brs, 1H, H5), 8.18 (s, 1H, Hα), 8.19 (d, *J* = 8.9 Hz, 1H, Ar8), 8.00 (d, *J* = 7.9 Hz, 1H, Ar5), 7.59-7.46 (m, 3H, Ar4/Ar6/Ar7), 7.16 (d, *J* = 8.1 Hz, 1H, Ar3), 4.80-4.68 (m, 3H, $OC\underline{H}_A\underline{H}_B$/H1'), 4.62-4.60 (m, 1H, H1'), 4.58-4.42 (m, 1H, H2'), 3.15-3.14 (m, 2H, H5'), 2.94-2.91 (m, 2H, H1'''), 2.72 (brs, 1H, H1''), 1.91-1.88 (m, 2H, H3'), 1.79-1.57 (m, 10H, H4'/H2'''/H3'''/H2''/H3''/H4''/H5''/H6''), 1.20-1.16 (m, 5H, H2''/H3''/H4''/H5''/H6''), 1.01 (d, *J* = 5.8 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 169.2 (C=O), 157.1 (C=N), 150.6 (Ar2), 149.3 (C4), 147.6 (Cβ), 132.7 (Ar8a), 130.2 (Ar4), 129.3 (Ar4a), 128.2 (Ar5), 128.1 (Ar7), 127.0 (Ar8), 125.7 (C5), 125.2 (Cα), 120.2 (Ar6), 119.3 (Ar3), 113.8 (Ar1), 67.7 ($O\underline{C}H_AH_B$), 55.7 (C1'), 49.1 (C2'), 40.4 (C5'), 37.9 (C2'''), 33.3 (C1''), 31.5 (C2''), 31.4 (C6''), 28.1 (C1'''), 27.5 (C3'), 25.16 (C3'''), 25.13 (C4''), 25.0 (C3''), 24.8 (C5''), 22.6 (C4'), 21.3

(C4'''/C5'''); IR (neat) $\bar{\nu}_{max}$ 3342, 3275, 3181, 2931, 2856, 1669, 1633, 1601, 1550, 1513, 1483, 1451, 1384, 1367, 1280, 1231, 1172, 1152, 1118, 1081, 1049, 860, 812, 778, 749, 668, 648, 588 cm^{-1}; MS (ESI +ve) *m/z* 615 ($[M - HCl + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{33}H_{47}N_{10}O_2$ 615.5300, found 615.3877 ($[M - HCl + H]^+$).

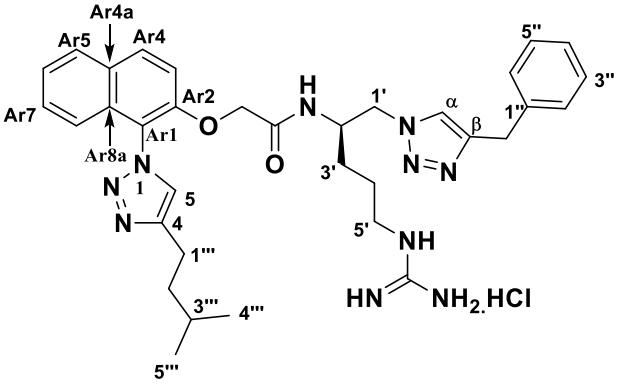
(*R*)-*N*-(1-(4-(Cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamide hydrochloride (30)



Following **General Procedure IV**, azide **64** (0.07 g, 0.09 mmol), 3-cyclohexyl-1-propyne (0.03 g, 0.27 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **142** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **142** (0.06 g, 0.06 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.36 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **30** (0.03 g, 66% over two steps) as a pale-yellow solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +74.1 (*c* 0.0056, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.19 (s, 1H, H5), 8.14 (d, *J* = 8.0 Hz, 1H, Ar8), 8.00 (t, *J* = 8.9 Hz, 1H, Ar5), 7.70-7.62 (m, 1H, Ar6), 7.61 (s, 1H, Hα), 7.58-7.45 (m, 1H, Ar7), 7.39 (d, *J* = 8.1 Hz, 1H, Ar4), 6.99 (d, *J* = 8.1 Hz, 1H, Ar3), 4.62 (s, 2H, $OC\underline{H}_A\underline{H}_B$), 4.48-4.36 (m, 1H, H1'), 4.36-4.24 (m, 1H, H1'), 4.22-4.08 (m, 1H, H2'), 3.08-2.96 (m, 2H, H5'), 2.80-2.70 (m, 2H, H1'''), 2.37 (d, *J* = 6.2 Hz, 2H, βC-$C\underline{H}_2$), 1.70-1.32 (m, 13H, H3'/H4'/H2'''/H3'''/H1''/H2''/H3''/H4''/H5''/H6''), 1.16-0.99 (m, 3H, H2''/

H4"/H6"), 0.92 (d, J = 2.4 Hz, 6H, H4'"/H5'"), 0.81-0.78 (m, 2H, H3"/H5"); ^{13}C NMR (101 MHz, CD_3OD) δ 167.8 (C=O), 157.4 (C=N), 150.9 (Ar2), 147.4 (C4), 145.2 (Cβ), 132.1 (Ar8a), 130.7 (Ar4), 128.96 (Ar4a), 128.93 (Ar5), 128.5 (Ar7), 125.9 (Ar8), 125.3 (C5), 123.7 (Cα), 121.3 (Ar6), 119.9 (Ar3), 115.2 (Ar1), 68.1 (O$\underline{C}H_AH_B$), 53.1 (C1'), 49.1 (C2'), 40.7 (C5', Observed by gHMBC), 38.5 (C2'"), 37.8 (βC-$\underline{C}H_2$), 33.0 (C1", Observed by gHMBC), 32.9 (C2"), 32.7 (C6"), 28.9 (C1'"), 27.6 (C3'), 26.4 (C3'"), 26.4 (C4", Observed by gHMBC), 26.0 (C3"), 25.4 (C5"), 23.5 (C4'), 22.8 (C4'"/C5'"); IR (neat) $\bar{\nu}_{max}$ 3339, 3273, 3177, 2953, 2927, 2854, 1907, 1668, 1634, 1601, 1549, 1514, 1483, 1450, 1383, 1367, 1350, 1281, 1231, 1221, 1153, 1117, 1083, 1052, 1025, 964, 919, 862, 811, 778, 750, 647, 608 cm^{-1}; MS (ESI +ve) *m/z* 629 ([M – HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{34}H_{49}N_{10}O_2$ 629.4034, found 629.4032 ([M – HCl + H]$^+$).

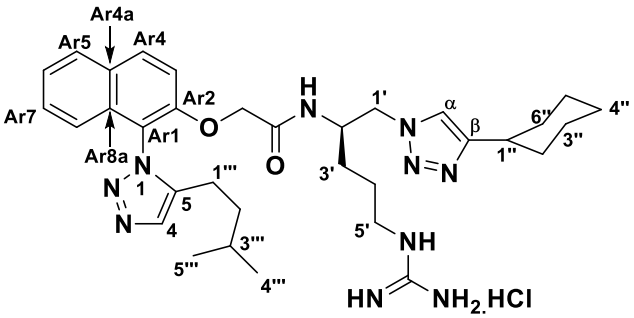
(*R*)-*N*-(1-(4-Benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamide hydrochloride (31)



Following **General Procedure IV**, azide **64** (0.07 g, 0.09 mmol), 3-phenyl-1-propyne (0.03 g, 0.27 mmol), $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **143** as a pale-yellow solid gum after flash chromatography over SiO_2 gel (MeOH/CH_2Cl_2 – 0:100 → 4:96). Following **General Procedure VII**, the intermediate **143** (0.06 g, 0.06 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.2 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the

amine salt **31** (0.028 g, 68% over two step) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +66.1 (*c* 0.0048, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (s, 1H, H5), 8.21 (s, 1H, Hα), 8.16 (d, *J* = 8.9 Hz, 1H, Ar8), 7.99 (d, *J* = 7.9 Hz, 1H, Ar5), 7.60-7.48 (m, 2H, Ar6/Ar7), 7.41 (d, *J* = 9.0 Hz, 1H, Ar4), 7.27-7.15 (m, 6H, Ar3/H2''/H3''/H4''/H5''/H6''), 4.78-4.72 (m, 1H, H1'), 4.67 (ABq, *J* = 17.6 Hz, 2H, OC$\underline{H}_A\underline{H}_B$), 4.60-4.52 (m, 1H, H1'), 4.42-4.34 (m, 1H, H2'), 4.08 (s, 2H, C$\underline{H}_2$Ph), 3.22-3.08 (m, 2H, H5'), 3.00-2.88 (m, 2H, H1'''), 1.84-1.46 (m, 7H, H3'/H4'/H2'''/H3'''), 0.99 (d, *J* = 5.7 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 169.1 (C=O), 157.1 (C=N), 150.6 (Ar2), 147.0 (C4), 144.4 (Cβ), 136.0 (C1''), 132.9 (Ar8a), 130.0 (Ar4), 129.2 (Ar4a), 128.9 (Ar5), 128.68 (C2''), 128.65 (C6''), 128.3 (C3''/C5''), 128.1 (Ar7), 127.8 (C4''), 127.0 (Ar8), 126.8 (C5), 125.3 (Cα), 120.1 (Ar6), 118.8 (Ar3), 113.7 (Ar1), 67.7 (O$\underline{C}H_AH_B$), 55.4 (C1'), 49.2 (C2'), 40.5 (C5'), 37.6 (C2'''), 34.9 ($\underline{C}H_2$Ph, Observed by gHMBC), 29.3 (C1'''), 28.1 (C3'), 27.5 (C3'''), 24.8 (C4'), 22.3 (C4'''/C5'''); IR (neat) $\bar{\nu}_{max}$ 3339, 3272, 3182, 3067, 2956, 2869, 1901, 1669, 1634, 1601, 1550, 1513, 1496, 1482, 1454, 1433, 1382, 1367, 1281, 1232, 1221, 1153, 1120, 1080, 1049, 967, 931, 863, 811, 778, 750, 721, 698, 674, 648 cm^{-1}; MS (ESI +ve) *m/z* 623 ([M – HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{34}H_{43}N_{10}O_2$ 623.3564, found 623.3561 ([M – HCl + H]$^+$).

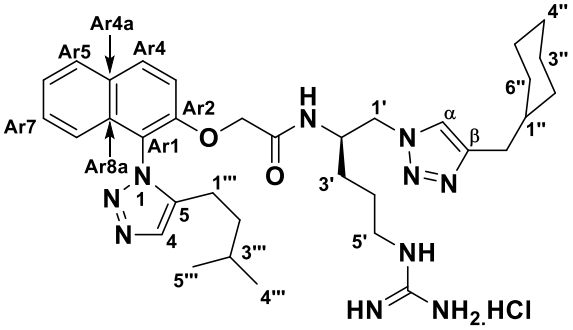
***N*-((*R*)-1-(4-Cyclohexyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamide hydrochloride (32)**



Following **General Procedure IV**, azide **66** (0.07 g, 0.09 mmol), cyclohexyl acetylene (0.03 g, 0.27 mmol), $CuSO_4{\cdot}5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **144** as a brown gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 4:96). Following **General Procedure VII**, the intermediate **144** (0.07 g, 0.08 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.6 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **32** (0.036 g, 52% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +65.6 (*c* 0.0062, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.41 (brs, 1H, H4), 8.34-8.16 (m, 2H, Hα/HAr8), 8.08-8.00 (m, 2H, Ar5/Ar4), 7.60-7.50 (m, 2H, Ar6/Ar7), 6.99 (d, *J* = 9.6 Hz, 1H, Ar3), 4.86-4.78 (m, 2H, O$\underline{C}H_A\underline{H}_B$), 4.70-4.56 (m, 2H, H1'), 4.44-4.32 (m, 1H, H2'), 3.05 (t, *J* = 7.0 Hz, 2H, H5'), 2.66-2.58 (m, 1H, H1''), 2.56-2.44 (m, 2H, H1'''), 2.04-1.50 (m, 10H, H4'/H2'''/H3'''/H2''/H3''/H4''/H5''/H6''), 1.48-1.06 (m, 7H, H3'/H2''/H3''/H4''/H5''/H6''), 0.69 (d, *J* = 7.0 Hz, 3H, H4'''), 0.67 (d, *J* = 7.1 Hz, 3H, H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 169.1 (C=O), 157.2 (C=N), 151.3 (Ar2), 142.9 (Cβ), 133.1 (C5), 131.7 (Ar8a), 130.5 (C4), 129.3 (Ar4), 128.9 (Ar4a), 128.3 (Ar5), 126.2 (Ar7), 125.3 (Ar8), 120.26 (Ar6), 120.23 (Cα), 117.7 (Ar3), 113.8 (Ar1), 67.6 (O$\underline{C}H_AH_B$), 55.9 (C1'), 49.3 (C2'), 40.6 (C5'), 36.5 (C2'''), 33.3 (C1''), 31.5 (C2''), 31.4 (C6''), 28.3 (C1'''), 27.0 (C3'), 25.2 (C3'''), 25.1 (C4''), 25.0 (C3''), 24.8 (C5''), 20.9 (C4'), 20.6 (C4'''/C5'''); IR (neat) $\bar{\nu}_{max}$ 3338, 3268, 3179, 3073, 2932, 2857,

2481, 1903, 1669, 1632, 1601, 1549, 1512, 1482, 1451, 1385, 1367, 1351, 1272, 1234, 1224, 1170, 1154, 1132, 1090, 1048, 990, 972, 941, 920, 858, 816, 750, 652 cm^{-1}; MS (ESI +ve) *m/z* 616 ([M – HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{33}H_{47}N_{10}O_2$ 615.3883, found 615.3873 ([M – HCl + H]$^+$).

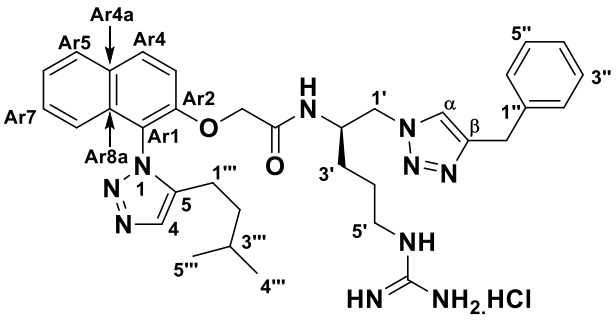
_N_-((_R_)-1-(4-(Cyclohexylmethyl)-1_H_-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-((1-(5-isopentyl-1_H_-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamide hydrochloride (33)



Following **General Procedure IV**, azide **66** (0.07 g, 0.09 mmol), 3-cyclohexyl-1-propyne (0.03 g, 0.27 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **145** as an off-white gum after flash chromatography over SiO_2 gel (MeOH/CH_2Cl_2 – 0:100 → 4:96). Following **General Procedure VII**, the intermediate **145** (0.07 g, 0.08 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.6 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **33** (0.03 g, 51% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +58.1 (*c* 0.0044, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (s, 1H, H4), 8.19 (s, 1H, Hα), 8.08-8.00 (m, 2H, Ar8/Ar5), 7.58-7.50 (m, 3H, Ar4/Ar6/Ar7), 6.99 (d, *J* = 9.6 Hz, 1H, Ar3), 4.78 (ABq, *J* = 19.0 Hz, 2H, OC$\underline{H}_A\underline{H}_B$), 4.67-4.54 (m, 2H, H1'/H2'), 4.44-4.30 (m, 1H, H1'), 3.26-3.20 (m, 1H, H1'''), 3.11-2.93 (m, 1H, H1'''), 2.67 (d, *J* = 12.8 Hz, 1H, βC-C$\underline{H}_2$), 2.54-2.44 (m, 3H, H5'/βC-C$\underline{H}_2$), 1.85-1.30 (m, 13H, H3'/H4'/H2'''/H3'''/H1''/H2''/H3''/H4''/H5''/H6''), 1.29-1.04 (m, 3H, H2''/H4''/H6''), 1.02-0.78 (m, 2H, H3''/H5''), 0.72-0.62 (m, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 168.8

(C=O), 157.0 (C=N), 151.0 (Ar2), 142.9 (C5), 132.9 (Ar8a), 131.6 (C4), 130.6 (Cβ), 129.4 (Ar4), 128.9 (Ar4a), 128.3 (Ar5), 126.8 (Ar7), 125.3 (Ar8), 120.3 (Ar6), 120.2 (Cα), 117.7 (Ar3), 113.8 (Ar1), 67.6 ($O\underline{C}H_AH_B$), 55.5 (C1'), 49.3 (C2'), 37.5 (C2'''), 36.5 (C5'), 33.9 (C1''), 33.0 (Cβ-$\underline{C}H_2$), 32.3 (C2''), 32.2 (C6''), 30.9 (C3'), 30.7 (3'''), 28.3 (C1'''), 28.1 (C4''), 27.0 (C3''), 25.7 (C5''), 25.7 (C4'), 25.6 (C4'''/5'''); IR (neat) $\bar{\nu}_{max}$ 3341, 3271, 3180, 3082, 2953, 2927, 2854, 2468, 1916, 1670, 1632, 1601, 1549, 1512, 1483, 1450, 1384, 1368, 1349, 1223, 1169, 1154, 1132, 1091, 1049, 1025, 991, 973, 935, 918, 863, 814, 780, 750, 678, 651 cm^{-1}; MS (ESI +ve) *m/z* 630 ($[M - HCl + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{34}H_{49}N_{10}O_2$ 629.4040, found 629.4059 ($[M - HCl + H]^+$).

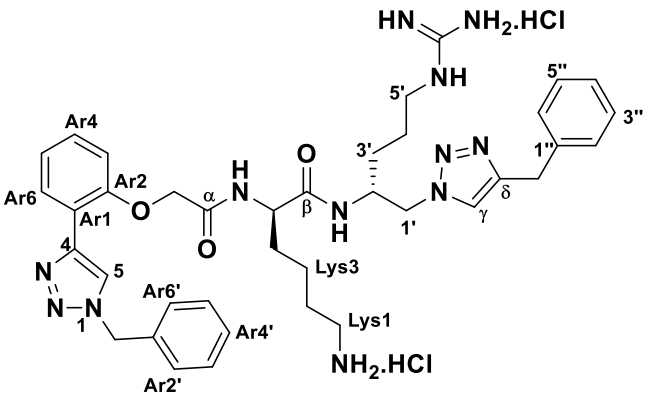
_N_-((_R_)-1-(4-Benzyl-1_H_-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-((1-(5-isopentyl-1_H_-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamide hydrochloride (34)



Following **General Procedure IV**, azide **66** (0.07 g, 0.09 mmol), 3-phenyl-1-propyne (0.03 g, 0.27 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **146** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 6:94). Following **General Procedure VII**, the intermediate **146** (0.07 g, 0.08 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.03 g, 1.6 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **34** (0.034 g, 57% over two steps) as a brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +73.7 (*c* 0.0051, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.23-8.08 (m, 3H, H4/Hα/Ar8), 8.02 (d, *J* = 8.2 Hz, 1H, Ar5), 7.60-7.38

(m, 3H, Ar4/Ar6/Ar7), 7.31-7.13 (m, 5H, H2''/H3''/H4''/H5''/H6''), 7.00 (d, *J* = 8.5 Hz, 1H, Ar3), 4.71 (ABq, *J* = 18.2 Hz, 2H, OC$\underline{H}_A\underline{H}_B$), 4.64-4.56 (m, 1H, H1'), 4.56-4.46 (m, 1H, H2'), 4.42-4.28 (m, 1H, H1'), 4.15 (s, 1H, C$\underline{H}_2$Ph), 4.01 (s, 1H, C$\underline{H}_2$Ph), 3.21-3.15 (m, 1H, H1'''), 3.10-2.99 (m, 1H, H1'''), 2.51 (m, 2H, H5'), 1.82-1.52 (m, 3H, H2'''/H3'''), 1.50-1.29 (m, 4H, H3'/H4'), 0.69 (d, *J* = 5.7 Hz, 3H, H4'''), 0.66 (d, *J* = 7.2 Hz, 3H, H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 167.5 (C=O), 157.8 (C=N), 151.8 (Ar2), 149.6 (C5), 140.4 (Ar8a), 138.5 (C1''), 133.3 (C4), 132.5 (Cβ), 130.3 (Ar4), 129.2 (Ar4a), 128.86 (C2''/C6''), 128.82 (Ar5), 128.74 (C3''/C5''), 128.71 (C4''), 128.6 (Ar7), 126.5 (Ar8), 125.3 (Ar6), 121.1 (Cα), 115.5 (Ar3), 115.4 (Ar1), 68.3 (O$\underline{C}H_AH_B$), 62.7 (C1'), 53.0 (C2'), 41.1 (C2'''), 40.8 (C5'), 36.8 ($\underline{C}H_2$Ph), 31.8 (C3'), 31.7 (3'''), 29.1 (C1'''), 25.3 (C4'), 22.1 (C4'''/5'''); IR (neat) $\bar{\nu}_{max}$ 3343, 3273, 3181, 3065, 2956, 2934, 2869, 2469, 1906, 1669, 1633, 1601, 1549, 1512, 1497, 1482, 1454, 1384, 1367, 1351, 1277, 1234, 1223, 1169, 1153, 1132, 1086, 1049, 992, 972, 934, 919, 863, 813, 780, 751, 722, 698, 652 cm^{-1}; MS (ESI +ve) *m/z* 624 ([M – HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{34}H_{43}N_{10}O_2$ 623.3570, found 623.3581 ([M – HCl + H]$^+$).

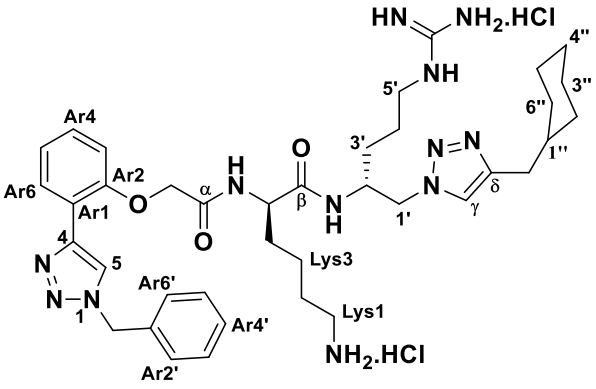
(*R*)-6-Amino-*N*-((*R*)-1-(4-benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(2-(1-benzyl-1*H*-1,2,3-triazol-4-yl)phenoxy)acetamido)hexanamide dihydrochloride (35)



Following **General Procedure IV**, azide **59** (0.07 g, 0.07 mmol), 3-phenyl-1-propyne (0.03 g, 0.22 mmol), $CuSO_4{\cdot}5H_2O$ (0.004 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.03 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **147** as an off-white solid after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 6:94). Following **General Procedure VII**, the intermediate **147** (0.06 g, 0.056 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.11 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **35** (0.03 g, 55% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +65.3 (*c* 0.0052, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (s, 1H, H5), 8.23-7.83 (m, 2H, Hγ/Ar4), 7.50-7.14 (m, 13H, Ar6/Ar5/Ar3/Ar2'/Ar3'/Ar4'/Ar5'/Ar6'/H2"/H3"/H4"/H5"/H6"), 5.69 (s, 2H, C$\underline{H}_2$Ph), 4.92-4.76 (m, 2H, OC$\underline{H}_A\underline{H}_B$), 4.84-4.70 (m, 2H, H1'), 4.40-4.32 (m, 1H, Lys5), 4.30-4.22 ((m, 1H, H2'), 4.03 (s, 2H, δC-C$\underline{H}_2$), 3.23-3.07 (m, 2H, H5'), 2.95-2.78 (m, 2H, Lys1), 1.80-1.50 (m, 8H, Lys4/H3'/Lys2 /H4'), 1.36-1.20 (m, 2H, Lys3); ^{13}C NMR (101 MHz, CD_3OD) δ 174.5 (βC=O), 169.7 (αC=O), 158.7 (C=N), 156.0 (Ar2), 143.1 (C4), 139.0 (Ar1'), 136.5 (C1"), 131.7 (Ar6), 130.3 (Cδ), 130.0 (C5), 129.9 (Ar4), 129.8 (C2"/C6"), 129.5 (Ar3'/Ar5'/C3"/C5"), 129.4 (Ar2'/Ar6'), 128.9 (Ar4'/C4"), 128.1 (Cγ), 123.4 (Ar5), 119.3 (Ar1), 114.4 (Ar3), 69.0 (O$\underline{C}H_AH_B$), 56.1 (C1'), 55.9 (Lys5), 55.0 ($\underline{C}H_2$Ph), 50.9 (C2'), 42.1 (C5'), 40.7 (Lys1),

32.5 (δC-$\underline{C}H_2$), 31.8 (Lys4), 30.0 (C3'), 28.1 (Lys2), 26.4 (C4'), 24.0 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3351, 3265, 3203, 3065, 2950, 2870, 1658, 1585, 1546, 1490, 1455, 1441, 1360, 1290, 1229, 1181, 1165, 1125, 1075, 1053, 978, 907, 840, 804, 758, 725, 698, 670, 644, 586 cm^{-1}; MS (ESI +ve) *m/z* 721 ($[M - 2HCl + H]^+$, 10%), 361 ($[M - 2HCl + H]^{2+}$, 100%); HRMS (ESI +ve TOF) calcd for $C_{38}H_{49}N_{12}O_3$ 721.4045, found 721.4046 ($[M - 2HCl + H]^+$).

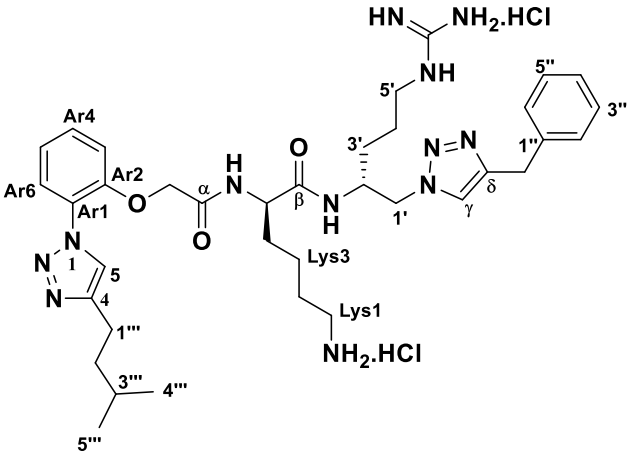
(*R*)-6-Amino-2-(2-(2-(1-benzyl-1*H*-1,2,3-triazol-4-yl)phenoxy)acetamido)-*N*-((*R*)-1-(4-(cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)hexanamide dihydrochloride (36)



Following **General Procedure IV**, azide **59** (0.09 g, 0.09 mmol), 3-cyclohexyl-1-propyne (0.03 g, 0.28 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.007 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **148** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 6:94). Following **General Procedure VII**, the intermediate **148** (0.06 g, 0.055 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.10 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **36** (0.025 g, 35% over two steps) as a pale-yellow solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +57.6 (*c* 0.0054, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.82 (s, 1H, H5), 8.25 (s, 1H, Hγ), 7.91 (apparent t, *J* = 7.0 Hz, 1H, Ar4), 7.44-7.36 (m, 6H, Ar6/Ar5/Ar2'/Ar3'/Ar5'/Ar6'), 7.16-7.10 (m, 2H, Ar4'/Ar3), 5.75 (s, 2H, C$\underline{H}_2$Ph), 4.88-4.70 (m, 3H, OC$\underline{H}_A\underline{H}_B$/H1'), 4.66-4.50 (m, 1H, H1'), 4.46-4.32 (m, 1H, Lys5), 4.28-4.19 (m, 1H, H2'), 3.24-3.10 (m, 2H,

δC-C$\underline{H}_2$), 2.92-2.84 (m, 2H, H5'), 2.66-2.60 (m, 2H, Lys1), 1.82-1.58 (m, 14H, Lys4/H3'/ Lys2/H4'/H1"/H2"/H3"/H4"/H5"/H6"), 1.44-1.28 (m, 2H, H2"/H6"), 1.26-1.12 (m, 3H, H3"/H4"/ H5"), 0.94-0.86 (m, 2H, Lys3); ^{13}C NMR (101 MHz, CD_3OD) δ 174.5 (βC=O), 171.6 (αC=O), 158.7 (C=N), 156.1 (Ar2), 145.1 (C4), 144.3 (Ar1'), 136.5 (Cδ), 131.8 (Ar6), 130.3 (C5/Ar4), 130.0 (Ar3'/Ar5'), 129.5 (Ar4'), 128.5 (Ar2'/Ar6'), 126.4 (Cγ), 123.3 (Ar5), 119.1 (Ar1), 114.4 (Ar3), 68.8 (O$\underline{C}H_AH_B$), 57.2 (C1'), 55.9 (Lys5), 55.3 ($\underline{C}H_2$Ph), 50.9 (C2'), 42.1 (C5'), 40.7 (Lys1), 38.9 (C1"), 33.9 (C2"/C6"), 33.8 (δC-$\underline{C}H_2$), 32.4 (Lys4), 32.3 (C4"), 29.7 (C3'), 28.1 (Lys2), 27.3 (C3"), 27.1 (C5"), 26.4 (C4'), 24.3 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3348, 3266, 3192, 3066, 2927, 2854, 2667, 1659, 1586, 1545, 1490, 1450, 1382, 1351, 1227, 1167, 1126, 1076, 1050, 977, 937, 911, 846, 805, 760, 725, 696, 675, 647, 585 cm^{-1}; MS (ESI +ve) *m/z* 727 ([M – 2HCl + H]$^+$, 20%), 364 ([M – 2HCl + H]$^{2+}$, 100%); HRMS (ESI +ve TOF) calcd for $C_{38}H_{55}N_{12}O_3$ 727.4514, found 727.4513 ([M – 2HCl + H]$^+$).

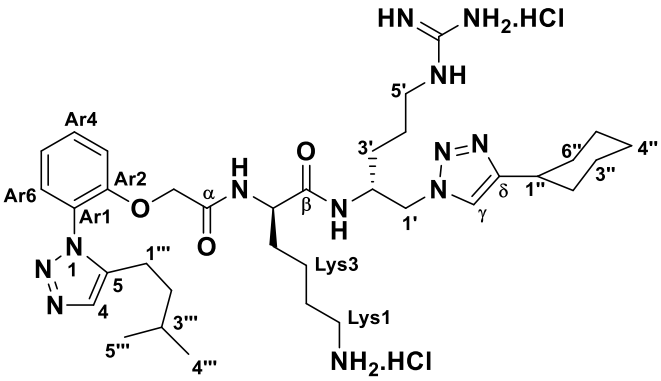
(*R*)-6-Amino-*N*-((*R*)-1-(4-benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(2-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamido)hexanamide dihydro chloride (37)



Following **General Procedure IV**, azide **61** (0.07 g, 0.07 mmol), 3-phenyl-1-propyne (0.03 g, 0.21 mmol), $CuSO_4{\cdot}5H_2O$ (0.004 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.02 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **149** as an off-white solid after flash chromatography over SiO_2 gel (MeOH/CH_2Cl_2 – 0:100

→ 5:95). Following **General Procedure VII**, the intermediate **149** (0.06 g, 0.06 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.10 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **37** (0.030 g, 56% over two steps) as a pale-yellow solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +68.1 (*c* 0.0052, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.54 (s, 1H, H5), 8.12 (s, 1H, Hγ), 7.65 (t, *J* = 7.5 Hz, 1H, Ar4), 7.54 (d, *J* = 8.0 Hz, 1H, Ar6), 7.32-7.20 (m, 7H, Ar5/Ar3/H2"/H3"/H4"/H5"/H6"), 4.84-4.74 (m, 2H, OC$\underline{H}_A\underline{H}_B$), 4.68-4.62 (m, 1H, H1'), 4.58-4.48 (m, 1H, H1'), 4.36-4.28 (m, 1H, Lys5), 4.20-4.12 (m, 1H, H2'), 4.12 (s, 2H, δC-C$\underline{H}_2$), 3.24-3.10 (m, 2H, H5'), 2.94-2.80 (m, 4H, Lys1/H1'"), 1.82-1.50 (m, 11H, H2'"/H3'"/H3'/Lys4/H4'/Lys2), 1.36-1.24 (m, 2H, Lys3), 0.96 (d, *J* = 5.0 Hz, 6H, H4'"/H5'"); ^{13}C NMR (101 MHz, CD_3OD) δ 173.9 (βC=O), 170.4 (αC=O), 158.4 (C=N), 152.0 (Ar2), 137.5 (Cδ), 133.5 (C4), 131.1 (C1"), 130.2 (C2"/C6"), 129.9 (C3"/C5"), 127.6 (C4"/Ar4), 127.4 (Ar6), 126.2 (C5), 125.9 (Cγ), 123.9 (Ar5), 123.3 (Ar1), 115.7 (Ar3), 69.7 (O$\underline{C}H_AH_B$), 57.6 (C1'), 54.9 (Lys5), 49.6 (C2'), 42.6 (C2'"), 40.7 (C5'), 38.5 (Lys1), 32.8 (δC-$\underline{C}H_2$), 31.6 (C3'), 29.8 (Lys4), 29.6 (C1'"), 28.6 (Lys2), 28.2 (C3'"), 25.8 (C4'), 23.2 (C4'"/C5'"), 22.8 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3375, 2956, 2932, 2870, 1653, 1572, 1548, 1508, 1462, 1381, 1367, 1288, 1254, 1228, 1150, 1113, 1058, 1005, 914, 883, 837, 793, 759, 721, 697, 670, 657 cm^{-1}; MS (ESI +ve) *m/z* 701 ([M – 2HCl + H]$^+$, 100%), 351 ([M – 2HCl + H]$^{2+}$, 40%); HRMS (ESI +ve TOF) calcd for $C_{36}H_{55}N_{12}O_3Cl_2$ 773.1236, found 773.1240 ([M + H]$^+$).

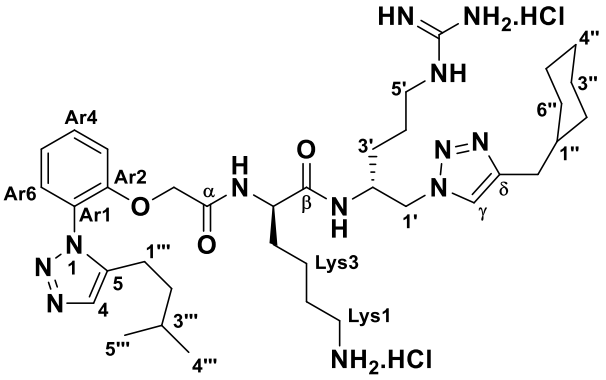
(*R*)-6-Amino-*N*-((*R*)-1-(4-cyclohexyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamido)hexanamide dihydrochloride (38)



Following **General Procedure IV**, azide **63** (0.02 g, 0.02 mmol), cyclohexylacetylene (0.007 g, 0.06 mmol), $CuSO_4 \cdot 5H_2O$ (0.001 g, 0.004 mmol) and sodium ascorbate (0.002 g, 0.008 mmol) were stirred in *t*-BuOH (1.2 mL) and H_2O (0.3 mL) for 16 h to give the intermediate **150** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 4:96). Following **General Procedure VII**, the intermediate **150** (0.02 g, 0.02 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.01 g, 0.40 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **38** (0.015 g, 98% over two steps) as an off-white solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +65.8 (*c* 0.0026, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (s, 1H, H4), 7.93 (s, 1H, Hγ), 7.68-7.60 (m, 1H, Ar4), 7.47 (d, *J* = 7.0 Hz, 1H, Ar6), 7.33 (t, *J* = 7.0 Hz, 1H, Ar5), 7.28-7.20 (m, 1H, Ar3), 4.80-4.68 (m, 3H, $OC\underline{H}_A\underline{H}_B$/H1'), 4.60-4.50 (m, 1H, H1'), 4.42-4.32 (m, 1H, Lys5), 4.16-4.08 (m, 1H, H2'), 3.28-3.14 (m, 2H, H1'''), 3.00-2.84 (m, 3H, H5'/H1''), 2.70-2.62 (m, 2H, Lys1), 1.88-1.56 (m, 13H, H2'''/H3'''/H3'/Lys4/H4'/Lys2/Lys3), 1.54-1.38 (m, 8H, H2''/H3''/H4''/H5''/H6''), 1.38-1.24 (m, 2H, H3''/H5''), 0.81 (d, *J* = 6.0 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 174.4 (βC=O), 170.2 (αC=O), 158.8 (C=N), 154.2 (Ar2), 143.4 (Cδ), 139.4 (C5), 134.0 (C4), 129.8 (Ar4), 127.1 (Ar6), 125.8 (Ar1), 123.56 (Cγ), 123.52 (Ar5), 115.5 (Ar3), 68.6 ($O\underline{C}H_AH_B$), 57.3 (C1'), 54.9 (Lys5), 50.9 (C2'), 42.1 (C2'''), 40.7 (C5'), 38.2 (Lys1), 35.1

(C1"), 33.3 (C3'), 32.6 (C2"/C6"), 29.7 (Lys4), 29.6 (Lys2), 28.8 (C3'"), 28.1 (C1'"), 26.9 (C4"), 26.7 (C3"/C5"), 24.2 (C4'), 23.6 (C4'"/C5'"), 22.7 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3378, 3350, 3265, 3066, 2932, 2864, 1915, 1664, 1603, 1548, 1508, 1464, 1452, 1384, 1367, 1286, 1272, 1250, 1230, 1167, 1114, 1090, 1057, 983, 956, 915, 892, 882, 837, 819, 802, 794, 761, 720, 667, 645, 628, 581 cm^{-1}; MS (ESI +ve) *m/z* 693 ($[M - 2HCl + H]^+$, 10%), 347 ($[M - 2HCl + H]^{2+}$, 100%); HRMS (ESI +ve TOF) calcd for $C_{35}H_{59}N_{12}O_3Cl_2$ 765.4210, found 765.4248 ($[M + H]^+$).

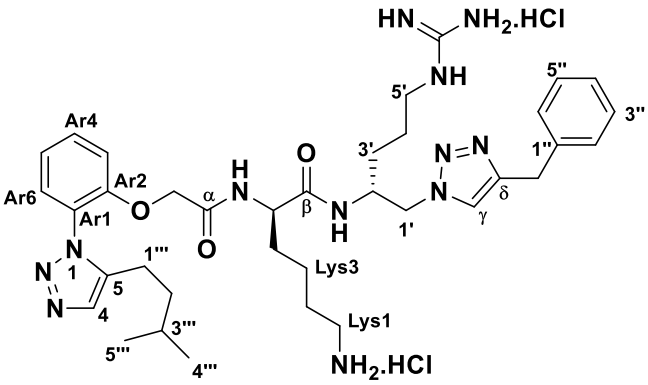
(*R*)-6-Amino-*N*-((*R*)-1-(4-(cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamido)hexanamide dihydrochloride (39)



Following **General Procedure IV**, azide **63** (0.10 g, 0.10 mmol), 3-cyclohexyl-1-propyne (0.04 g, 0.32 mmol), $CuSO_4 \cdot 5H_2O$ (0.005 g, 0.02 mmol) and sodium ascorbate (0.008 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **151** as an off-white solid after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 4:96). Following **General Procedure VII**, the intermediate **151** (0.07 g, 0.06 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.32 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **39** (0.050 g, 64% over two steps) as an off-white solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +61.4 (*c* 0.0048, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.43 (s, 1H, H4), 8.18 (s, 1H, Hγ), 7.67 (apparent t, *J* = 8.9 Hz, 1H, Ar4), 7.51 (d, *J* = 9.1 Hz, 1H, Ar6), 7.35 (d, *J*

= 9.1 Hz, 1H, Ar5), 7.26 (t, *J* = 8.9 Hz, 1H, Ar3), 4.74-4.60 (m, 3H, OC$\underline{H}_A\underline{H}_B$/H1'), 4.51-4.43 (m, 1H, H1'), 4.38-4.30 (m, 1H, Lys5), 4.14-4.08 (m, 1H, H2'), 3.22-3.13 (m, 2H, H1'"), 2.96-2.87 (m, 2H, H5'), 2.66-2.60 (m, 4H, δC-C$\underline{H}_2$/Lys1), 1.78-1.54 (m, 14H, H1"/Lys4/Lys2 /Lys3/H3'/H4'/H2'"/H3'"), 1.50-1.36 (m, 5H, H2"/H3"/H4"/H5"/H6"), 1.33-1.12 (m, 3H, H2"/H4"/ H6"), 1.05-0.91 (m, 2H, H3"/H5"), 0.80 (d, *J* = 6.4 Hz, 6H, H4'"/H5'"); ^{13}C NMR (101 MHz, CD_3OD) δ 172.9 (βC=O), 168.5 (αC=O), 157.2 (C=N), 152.5 (Ar2), 152.4 (Cδ), 142.9 (C5), 132.9 (C4), 129.3 (Ar4), 128.3 (Ar6), 127.8 (Ar1), 123.7 (Cγ), 121.9 (Ar5), 114.0 (Ar3), 67.1 (O$\underline{C}H_AH_B$), 56.3 (C1'), 53.4 (Lys5), 49.4 (C2'), 40.5 (C2'"), 39.2 (C5'), 37.3 (Lys1), 36.4 (C1"), 32.3 (δC-$\underline{C}H_2$), 32.2 (C2"), 31.0 (C6"), 30.4 (C3'), 28.1 (Lys4), 27.2 (Lys2), 26.5 (C3'"), 25.7 (C1'"), 25.6 (C4"), 24.8 (C3"/C5"), 22.6 (C4'), 21.1 (C4'"/C5'"), 20.9 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3351, 3268, 3195, 3066, 2927, 2856, 2667, 2067, 1916, 1662, 1543, 1508, 1465, 1451, 1386, 1368, 1290, 1229, 1168, 1129, 1051, 985, 958, 936, 916, 846, 762, 668, 646, 585 cm^{-1}; MS (ESI +ve) *m/z* 707 ([M – 2HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{36}H_{59}N_{12}O_3$ 707.4827, found 707.4824 ([M – 2HCl + H]$^+$).

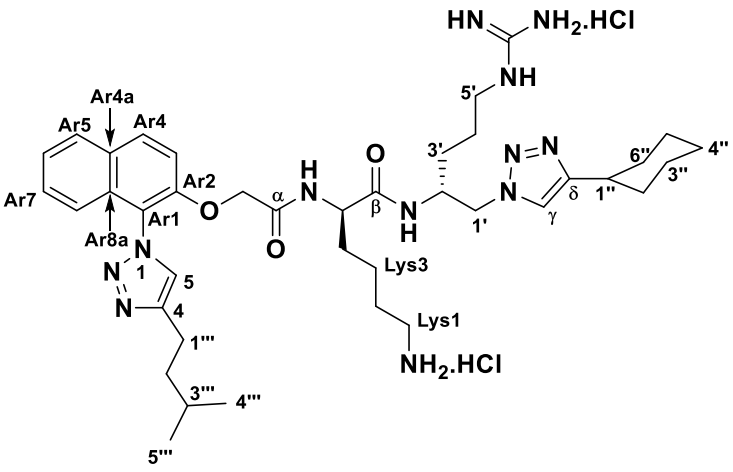
(*R*)-6-Amino-*N*-((*R*)-1-(4-benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-(2-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)phenoxy)acetamido)hexanamide dihydrochloride (40)



Following **General Procedure IV**, azide **63** (0.03 g, 0.03 mmol), 3-phenyl-1-propyne (0.01 g, 0.09 mmol), $CuSO_4 \cdot 5H_2O$ (0.002 g, 0.006 mmol) and sodium ascorbate (0.003 g, 0.012 mmol) were stirred in *t*-BuOH (1.5 mL) and

H_2O (0.3 mL) for 16 h to give the intermediate **152** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 4:96). Following **General Procedure VII**, the intermediate **152** (0.03 g, 0.03 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.01 g, 0.56 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **40** (0.012 g, 52% over two steps) as an off-white solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +66.5 (*c* 0.0028, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.16 (s, 1H, H4), 7.64 (s, 1H, Hγ), 7.63 (apparent t, *J* = 7.0 Hz, 1H, Ar4), 7.48-7.41 (m, 1H, Ar6), 7.36-7.20 (m, 7H, Ar5/Ar3/H2"/H3"/H4"/H5"/H6"), 4.80-4.58 (m, 3H, OC$\underline{H}_A\underline{H}_B$/H1'), 4.57-4.44 (m, 1H, H1'), 4.36-4.28 (m, 1H, Lys5), 4.20-4.08 (m, 3H, H2'/H1'"), 3.26-3.14 (m, 2H, H5'), 2.92-2.82 (m, 2H, C$\underline{H}_2$Ph), 2.68-2.60 (m, 2H, Lys1), 1.80-1.36 (m, 11H, Lys4/Lys2/H3'/H4'/H2'"/H3'"), 1.30-1.20 (m, 2H, Lys3), 0.79 (d, *J* = 5.5 Hz, 3H, H4'"), 0.76 (d, *J* = 6.0 Hz, 3H, H5'"); ^{13}C NMR (101 MHz, CD_3OD) δ 174.2 (βC=O), 170.0 (αC=O), 158.7 (C=N), 154.1 (Ar2), 154.0 (Cδ), 138.4 (C5), 134.1 (C1"), 130.1 (C4), 130.0 (Ar4), 129.9 (C2"/C6"), 129.89 (Ar6), 129.86 (C3"/C5"), 128.3 (C4"), 125.7 (Ar1), 123.5 (Cγ), 115.4 (Ar5), 111.5 (Ar3), 68.6 (O$\underline{C}H_AH_B$), 56.4 (C1'), 54.8 (Lys5), 50.8 (C2'), 42.1 (C2'"), 40.6 (C5'), 38.1 (Lys1), 32.5 ($\underline{C}H_2$Ph), 31.4 (C3'), 29.9 (Lys4), 29.8 (Lys2), 28.7 (C3'"), 28.1 (C1'"), 26.4 (C4'), 23.9 (Lys3), 22.7 (C4'"/C5'"); IR (neat) $\bar{\nu}_{max}$ 3374, 3075, 2956, 2932, 2870, 1654, 1572, 1548, 1508, 1462, 1381, 1367, 1288, 1253, 1229, 1150, 1113, 1058, 1005, 914, 883, 838, 793, 759, 720, 697, 670, 657 cm^{-1}; MS (ESI +ve) *m/z* 701 ([M – 2HCl + H]$^+$, 70%), 351 ([M – 2HCl + H]$^{2+}$, 100%); HRMS (ESI +ve TOF) calcd for $C_{36}H_{55}N_{12}O_3Cl_2$ 773.3897, found 773.3914 ([M + H]$^+$).

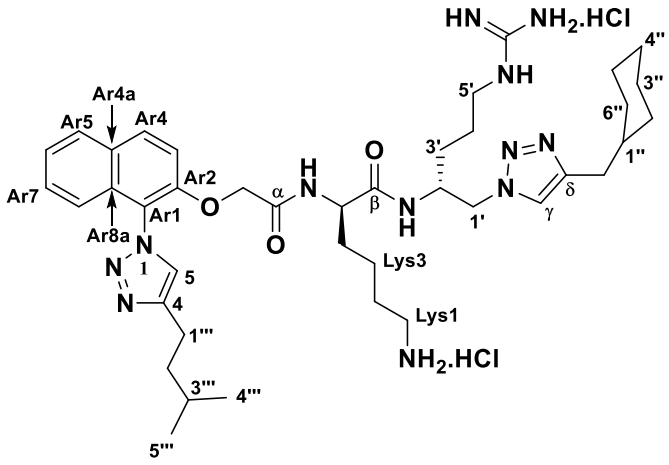
(*R*)-6-Amino-*N*-((*R*)-1-(4-cyclohexyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido)hexanamide dihydrochloride (41)



Following **General Procedure IV**, azide **65** (0.08 g, 0.08mmol), cyclohexylacetylene (0.03 g, 0.24 mmol), $CuSO_4{\cdot}5H_2O$ (0.004 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.03 mmol) were stirred in *t*-BuOH (2.0 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **153** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 8:92). Following **General Procedure VII**, the intermediate **153** (0.06 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.00 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **41** (0.03 g, 46% over two steps) as an off-white solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +59.1 (*c* 0.0052, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.30 (s, 1H, H5), 8.29 (s, 1H, Hγ), 8.18 (d, *J* = 9.2 Hz, 1H, Ar8), 7.98 (d, *J* = 7.5 Hz, 1H, Ar5), 7.61 (ddd, *J* = 9.2, 9.2, 1.7 Hz, 1H, Ar7), 7.57-7.49 (m, 2H, Ar6/Ar4), 7.14 (d, *J* = 8.3 Hz, 1H, Ar3), 4.93-4.89 (m, 2H, $OC\underline{H}_A\underline{H}_B$), 4.77-4.72 (m, 1H, H1'), 4.59-4.53 (m, 1H, H1'), 4.37-4.32 (m, 1H, Lys5), 4.12-4.09 (m, 1H, H2'), 3.18-3.14 (m, 2H, H5'), 2.95-2.91 (m, 2H, Lys1), 2.84-2.78 (m, 3H, H1'''/H1''), 2.00-1.96 (m, 2H, Lys4), 1.74-1.60 (m, 14H, H2'''/H3'''/Lys2/H3'/H4'/H2''/H3''/H4''/H5''/H6''), 1.48-1.21 (m, 7H, Lys3/H2''/H3''/H4''/H5''/H6''), 1.01 (d, *J* = 6.2 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 173.0 (βC=O), 169.1 (αC=O), 157.1 (C=N), 150.8 (Ar2), 148.7 (C4), 147.6 (Cδ), 132.5 (Ar8a), 130.3 (Ar4),

129.1 (Ar4a), 128.6 (Ar5), 128.0 (Ar7), 126.9 (Ar8), 125.5 (C5), 125.1 (Cγ), 120.2 (Ar6), 119.1 (Ar3), 113.9 (Ar1), 67.4 (O<u>C</u>H_AH_B), 55.7 (C1'), 53.5 (Lys5), 49.3 (C2'), 40.4 (C5'), 39.0 (Lys1), 37.9 (C2'''), 33.4 (C1''), 31.6 (C2''), 31.5 (C6''), 30.9 (Lys4), 28.1 (Lys2), 27.5 (C1'''), 26.5 (C3'), 25.2 (C4''), 25.0 (C3''/C5''), 24.8 (C3'''), 22.6 (C4'), 22.5 (C4'''/C5'''), 21.3 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3348, 3265, 3202, 3066, 2932, 2860, 1662, 1544, 1514, 1483, 1451, 1384, 1366, 1349, 1279, 1220, 1168, 1117, 1081, 1049, 816, 749, 668, 585 cm^{-1}; MS (ESI +ve) *m/z* 743 ($[M - 2HCl + H]^+$, 60%), 372 ($[M - 2HCl + H]^{2+}$, 100%); HRMS (ESI +ve TOF) calcd for $C_{39}H_{59}N_{12}O_3$ 743.4833, found 743.4866 ($[M - 2HCl + H]^+$).

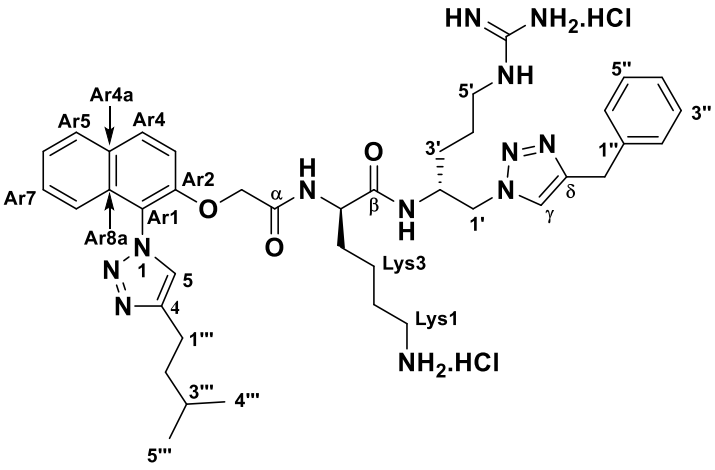
(*R*)-6-Amino-*N*-((*R*)-1-(4-(cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido) hexanamide dihydrochloride (42)



Following **General Procedure IV**, azide **65** (0.07 g, 0.07 mmol), 3-cyclohexyl-1-propyne (0.03 g, 0.21 mmol), $CuSO_4 \cdot 5H_2O$ (0.004 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.02 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **154** as a light brown waxy solid after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **154** (0.05 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 0.10 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (2 mL) to give the amine salt **42** (0.03 g, 52% over two steps) as a pale brown solid that rapidly transitioned to a sticky

gum. $[\alpha]_D^{23}$ +65.3 (*c* 0.0052, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (s, 2H, H5/Hγ), 8.13 (d, *J* = 8.2 Hz, 1H, Ar8), 7.90 (d, *J* = 7.5 Hz, 1H, Ar5), 7.55 (ddd, *J* = 8.2, 8.2, 1.6 Hz, 1H, Ar7), 7.52-7.40 (m, 2H, Ar6/Ar4), 7.08 (d, *J* = 7.5 Hz, 1H, Ar3), 4.88-4.80 (m, 2H, $OC\underline{H}_A\underline{H}_B$), 4.76-4.66 (m, 1H, H1'), 4.56-4.46 (m, 1H, H1'), 4.34-4.26 (m, 1H, Lys5), 4.10-4.02 (m, 1H, H2'), 3.16-3.06 (m, 2H, H5'), 2.90-2.82 (m, 2H, Lys1), 2.78-2.74 (m, 2H, H1'''), 2.62-2.56 (m, 3H, H1''/δC-$C\underline{H}_2$), 1.80-1.50 (m, 18H, Lys4/Lys2/Lys3/H2'''/H3'''/H3'/H4'/ H2''/H3''/H4''/ H5''/H6''), 1.26-1.08 (m, 5H, H2''/H3''/H4''/H5''/H6''), 0.95 (d, *J* = 4.0 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 172.9 (βC=O), 169.1 (αC=O), 157.1 (C=N), 150.8 (Ar2), 147.6 (C4), 143.4 (Cδ), 132.5 (Ar8a), 130.4 (Ar4), 129.1 (Ar4a), 128.6 (Ar5), 128.0 (Ar7), 127.0 (Ar8), 126.8 (C5), 125.1 (Cγ), 120.3 (Ar6), 119.1 (Ar3), 113.9 (Ar1), 67.5 ($OC\underline{H}_AH_B$), 65.5 (C1'), 55.7 (Lys5), 53.5 (C2'), 49.3 (C5'), 40.5 (Lys1), 39.0 (C2'''), 38.0 (C1''), 37.3 (δC-$C\underline{H}_2$), 32.3 (C2''), 32.2 (C6''), 30.8 (Lys4), 30.7 (Lys2), 28.1 (C4''), 27.5 (C1'''), 26.5 (C3'), 25.7 (C3''), 25.6 (C5''), 24.8 (C3'''), 22.7 (C4'), 22.6 (C4'''/C5'''), 21.4 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3346, 3266, 3193, 3063, 2950, 2927, 2855, 1664, 1602, 1543, 1514, 1483, 1451, 1383, 1367, 1349, 1280, 1219, 1168, 1154, 1117, 1080, 1050, 963, 933, 916, 863, 815, 779, 749, 673, 648, 586 cm^{-1}; MS (ESI +ve) *m/z* 757 ($[M - 2HCl + H]^+$, 50%), 379 ($[M - 2HCl + H]^{2+}$, 100%); HRMS (ESI +ve TOF) calcd for $C_{40}H_{61}N_{12}O_3$ 757.4990, found 757.4969 ($[M - 2HCl + H]^+$).

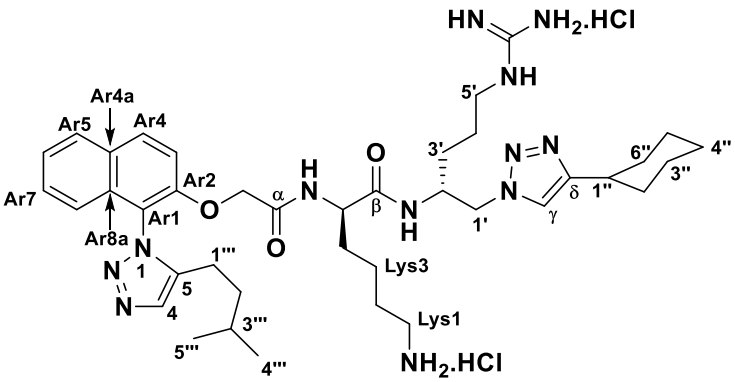
(*R*)-6-Amino-*N*-((*R*)-1-(4-benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(4-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido)hexanamide dihydrochloride (43)



Following **General Procedure IV**, azide **65** (0.07 g, 0.07 mmol), 3-phenyl-1-propyne (0.02 g, 0.21 mmol), $CuSO_4{\cdot}5H_2O$ (0.004 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.02 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **155** as a pale brown solid after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 6:94). Following **General Procedure VII**, the intermediate **155** (0.060 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.08 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **43** (0.03 g, 52% over two steps) as a brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +57.3 (*c* 0.0042, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.41 (brs, 1H, H5), 8.28-8.16 (m, 2H, H2''/H6''), 7.99 (brs, 1H, Hγ), 7.66-7.48 (m, 4H, Ar8/Ar5/Ar7/Ar6), 7.34-7.22 (m, 4H, H3''/H5''/Ar4/Ar3), 7.20-7.14 (m, 1H, H4''), 4.92-4.80 (m, 2H, $OC\underline{H}_A\underline{H}_B$), 4.74-4.66 (m, 1H, H1'), 4.60-4.52 (m, 1H, H1'), 4.42-4.32 (m, 1H, Lys5), 4.28-4.14 (m, 3H, $C\underline{H}_2$Ph/H2'), 3.22-3.12 (m, 2H, H5'), 3.02-2.84 (m, 2H, Lys1), 2.84-2.76 (m, 2H, H1'''), 1.82-1.52 (m, 10H, Lys4/Lys2/H3'/H4'/H2'''), 1.50-1.42 (m, 1H, H3'''), 1.34-1.20 (m, 2H, Lys3), 1.01 (d, *J* = 4.8 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 172.0 (βC=O), 168.1 (αC=O), 156.3 (C=N), 150.0 (Ar2), 149.9 (C4), 143.7 (Cδ), 135.5 (C1''), 132.0 (Ar8a), 129.4 (Ar4), 128.9 (Ar4a), 128.3 (Ar5), 128.0 (Ar7), 127.8 (C2''/C6''),

127.6 (C3"/C5"), 127.2 (Ar8), 126.1 (C5), 125.8 (C4"), 124.3 (Cγ), 119.3 (Ar6), 118.0 (Ar3), 113.1 (Ar1), 66.8 (O$\underline{C}H_AH_B$), 64.7 (C1'), 54.5 (Lys5), 52.6 (C2'), 48.5 (C5'), 39.7 (Lys1), 38.2 (C2'"), 36.9 ($\underline{C}H_2$Ph), 30.1 (Lys4), 28.7 (Lys2), 27.4 (C1'"), 26.7 (C3'), 25.7 (C3'"), 24.0 (C4'), 21.6 (C4'"/C5'"), 20.6 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3349, 3266, 3195, 3063, 2954, 2869, 1662, 1602, 1545, 1515, 1497, 1484, 1454, 1382, 1367, 1347, 1279, 1232, 1221, 1166, 1153, 1116, 1079, 1050, 969, 926, 909, 865, 812, 779, 750, 722, 699, 675, 667, 647 cm^{-1}; MS (ESI +ve) *m/z* 751 ($[M - 2HCl + H]^+$, 20%), 376 ($[M - 2HCl + H]^{2+}$, 100%); HRMS (ESI +ve TOF) calcd for $C_{40}H_{56}N_{12}O_3Cl$ 787.4287, found 787.4312 ($[M - HCl + H]^+$).

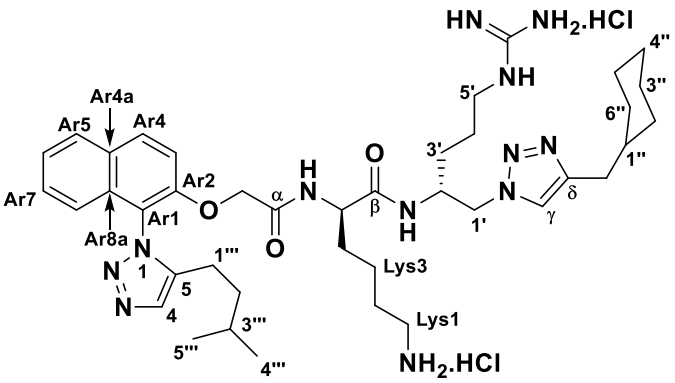
(2*R*)-6-Amino-*N*-((*R*)-1-(4-cyclohexyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido)hexanamide dihydrochloride (44)



Following **General Procedure IV**, azide **67** (0.08 g, 0.08 mmol), cyclohexylacetylene (0.03 g, 0.24 mmol), $CuSO_4 \cdot 5H_2O$ (0.004 g, 0.02 mmol) and sodium ascorbate (0.006 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **156** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **156** (0.07 g, 0.06 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.27 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **44** (0.036 g, 55% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +71.9 (*c* 0.0052, MeOH). ^{1}H

NMR (400 MHz, CD_3OD) δ 8.25 (s, 1H, H4), 8.25-8.21 (m, 1H, Ar8), 7.99 (s, 1H, Hγ), 8.03-7.97 (m, 1H, Ar5), 7.64 (ddd, *J* = 9.2, 2.3 Hz, 1H, Ar7), 7.56-7.50 (m, 2H, Ar6/Ar4), 6.99-6.95 (m, 1H, Ar3), 5.01-4.65 (m, 3H, $OC\underline{H}_A\underline{H}_B$/H1'), 4.58-4.48 (m, 1H, H1'), 4.40-4.28 (m, 1H, Lys5), 4.12-4.04 (m, 1H, H2'), 3.23-3.12 (m, 2H, H5'), 3.00-2.76 (m, 2H, H1'''), 2.72-2.63 (m, 1H, H1''), 2.54-2.44 (m, 2H, Lys1), 2.08-1.91 (m, 2H, Lys4), 1.90-1.08 (m, 21H, H2'''/H3'''/H3'/H4'/Lys2/Lys3/H2''/H3''/H4''/H5''/H6''), 0.71-0.66 (m, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 176.7 (βC=O), 172.6 (αC=O), 161.0 (C=N), 155.3 (Ar2), 146.0 (Cδ), 136.7 (C5), 136.6 (Ar8a), 135.3 (C4), 134.7 (Ar4), 133.3 (Ar4a), 132.5 (Ar5), 132.1 (Ar7), 129.0 (Ar8), 128.1 (Ar6), 124.2 (Cγ), 121.2 (Ar3), 118.0 (Ar1), 71.3 ($O\underline{C}H_AH_B$), 58.8 (C1'), 57.4 (Lys5), 53.2 (C2'), 44.4 (C2'''), 43.0 (C5'), 40.5 (Lys1), 37.9 (C1''), 36.0 (Lys4), 35.9 (C3'), 34.9 (Lys2), 30.9 (C2''), 30.5 (C6''), 29.4 (C4''), 29.3 (C3''), 29.2 (C5''), 28.7 (C3'''), 26.4 (1'''), 26.3 (C4'), 24.9 (C4'''/C5'''), 24.4 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3343, 3264, 3185, 3063, 2931, 2860, 1663, 1601, 1541, 1513, 1483, 1451, 1385, 1367, 1347, 1278, 1220, 1170, 1154, 1131, 1082, 1049, 990, 973, 931, 919, 859, 818, 779, 750, 678, 652 cm^{-1}; MS (ESI +ve) *m/z* 744 ($[M - 2HCl + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{39}H_{59}N_{12}O_3$ 743.4833, found 743.4839 ($[M - 2HCl + H]^+$).

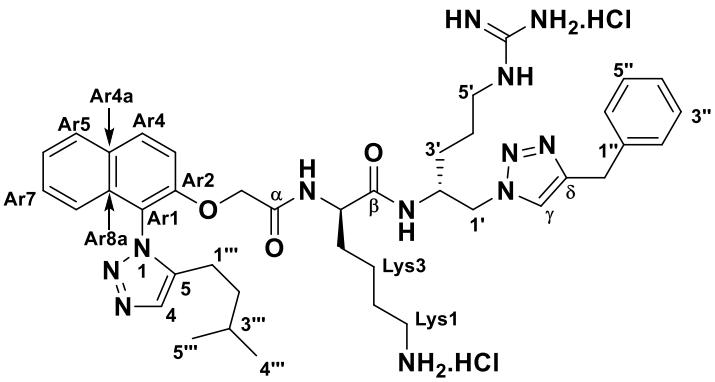
(2*R*)-6-Amino-*N*-((*R*)-1-(4-(cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido) hexanamide dihydrochloride (45)



Following **General Procedure IV**, azide **67** (0.08 g, 0.08 mmol), 3-cyclohexyl-1-propyne (0.03 g, 0.24 mmol), $CuSO_4 \cdot 5H_2O$ (0.004 g, 0.02 mmol) and sodium ascorbate (0.006 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **157** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **157** (0.06 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.05 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **45** (0.036 g, 54% over two steps) as a brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +66.1 (*c* 0.0050, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.33 (s, 1H, H4), 8.29 (s, 1H, Hγ), 8.25-8.21 (m, 1H, Ar8), 8.06-8.00 (m, 1H, Ar5), 7.64 (ddd, *J* = 9.2, 9.2, 2.3 Hz, 1H, Ar7), 7.58-7.49 (m, 2H, Ar6/Ar4), 7.00-6.94 (m, 1H, Ar3), 5.04-4.68 (m, 3H, $OC\underline{H}_A\underline{H}_B$/H1'), 4.62-4.48 (m, 1H, H1'), 4.40-4.28 (m, 1H, Lys5), 4.12-4.04 (m, 1H, H2'), 3.23-3.10 (m, 2H, H5'), 3.01-2.85 (m, 1H, H1'''), 2.77-2.58 (m, 3H, H1'''/Lys1), 2.54-2.44 (m, 2H, δC-C$\underline{H}_2$), 1.85-1.49 (m, 14H, H2'''/H3'''/H3'/H4'/Lys2/Lys3/Lys4/H1''), 1.46-1.08 (m, 8H, H2''/H3''/H4''/H5''/H6''), 1.03-0.80 (m, 2H, H3''/H5''), 0.74-0.64 (m, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 171.5 (βC=O), 167.3 (αC=O), 157.9 (C=N), 151.9 (Ar2), 145.5 (Cδ), 141.1 (C5), 132.4 (Ar8a), 131.7 (C4), 131.4 (Ar4), 129.2 (Ar4a), 128.7 (Ar5), 128.5 (Ar7), 125.3 (Ar8), 123.4 (Ar6), 121.1 (Cγ), 118.4 (Ar3),

115.5 (Ar1), 68.4 (O<u>C</u>H_AH_B), 53.1 (C1'), 49.5 (Lys5), 39.1 (C2'), 37.86 (C2'''), 37.83 (C5'), 36.9 (Lys1), 32.99 (δC-<u>C</u>H_2), 32.92 (C1''), 31.8 (Lys4), 29.1 (C3'), 27.1 (Lys2), 26.98 (C2''), 26.95 (C6''), 26.4 (C4''), 26.03 (C3''), 26.01 (C5''), 25.3 (C3'''), 22.5 (C1'''), 22.4 (C4'), 22.2 (C4'''/C5'''), 20.7 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3345, 3264, 3187, 3061, 2952, 2927, 2855, 2666, 1663, 1634, 1602, 1542, 1513, 1483, 1450, 1385, 1368, 1347, 1279, 1220, 1168, 1154, 1131, 1082, 1050, 1025, 992, 972, 932, 863, 818, 780, 749, 680, 651 cm^{-1}; MS (ESI +ve) *m/z* 758 ($[M - 2HCl + H]^+$, 70%), 380 ($[M - 2HCl + H]^{2+}$, 100%); HRMS (ESI +ve TOF) calcd for $C_{40}H_{61}N_{12}O_3$ 757.4990, found 757.5007 ($[M - 2HCl + H]^+$).

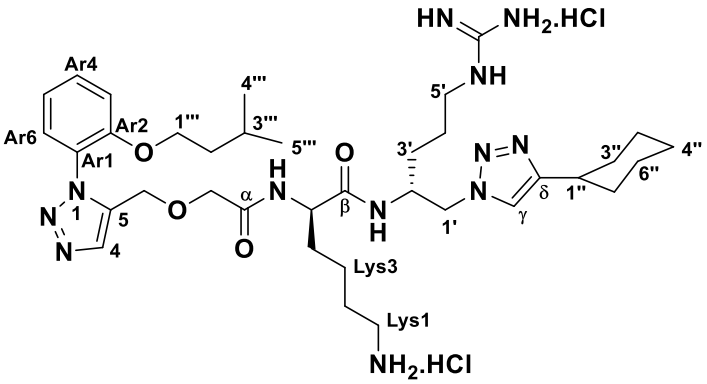
(2*R*)-6-Amino-*N*-((*R*)-1-(4-benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(5-isopentyl-1*H*-1,2,3-triazol-1-yl)naphthalen-2-yl)oxy)acetamido)hexanamide dihydrochloride (46)



Following **General Procedure IV**, azide **67** (0.08 g, 0.08 mmol), 3-phenyl-1-propyne (0.03 g, 0.24 mmol), $CuSO_4 \cdot 5H_2O$ (0.004 g, 0.02 mmol) and sodium ascorbate (0.006 g, 0.04 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **158** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 6:94). Following **General Procedure VII**, the intermediate **158** (0.07 g, 0.06 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.27 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **46** (0.035 g, 53% over two steps) as a white solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +58.9 (*c* 0.0052, MeOH). ^{1}H NMR (400

MHz, CD_3OD) δ 8.25-8.18 (m, 1H, Ar8), 8.03-7.93 (m, 2H, Ar5/Ar7), 7.95 (s, 1H, H4), 7.63-7.50 (m, 3H, Ar6/Ar4/Hγ), 7.33-7.17 (m, 5H, H2"/H3"/H4"/H5"/H6"), 6.99-6.95 (m, 1H, Ar3), 4.99-4.68 (m, 2H, $OC\underline{H}_A\underline{H}_B$), 4.62-4.55 (m, 1H, H1'), 4.49-4.39 (m, 1H, H1'), 4.36-4.27 (m, 1H, Lys5), 4.17-4.02 (m, 3H, H2'/δC-$C\underline{H}_2$), 3.18-3.12 (m, 2H, H5'), 2.92-2.84 (m, 1H, H1'"), 2.77-2.67 (m, 1H, H1'"), 2.58-2.42 (m, 2H, Lys1), 1.77-1.44 (m, 8H, H3'/H4'/Lys2/Lys4), 1.43-1.30 (m, 3H, H2'"/H3'"), 1.28-1.00 (m, 2H, Lys3), 0.71-0.66 (m, 6H, H4'"/H5'"); ^{13}C NMR (101 MHz, CD_3OD) δ 171.5 (βC=O), 167.2 (αC=O), 157.9 (C=N), 151.8 (Ar2), 146.0 (Cδ), 141.2 (C5), 141.1 (Ar8a), 140.0 (C4), 132.0 (C1"), 131.7 (Ar4), 131.4 (Ar4a), 129.2 (C2"), 128.9 (C6"), 128.79 (C3"), 128.74 (C5"), 128.5 (Ar5), 126.48 (Ar7), 126.46 (Ar8), 125.3 (Ar6), 125.2 (C4"), 123.4 (Cγ), 121.1 (Ar3), 115.5 (Ar1), 68.4 ($O\underline{C}H_AH_B$), 53.0 (C1'), 49.5 (Lys5), 39.1 (C2'), 36.9 (C2'"), 31.8 (δC-$\underline{C}H_2$), 29.1 (C5'), 27.1 (Lys1), 26.99 (Lys4), 26.96 (C3'), 25.3 (Lys2), 22.5 (C3'"), 22.4 (1'"), 22.23 (C4'), 22.20 (C4'"/C5'"), 20.7 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3342, 3264, 3190, 3064, 2955, 2935, 2869, 1662, 1601, 1543, 1513, 1483, 1455, 1384, 1367, 1346, 1279, 1221, 1170, 1154, 1131, 1080, 1049, 991, 972, 930, 864, 815, 780, 750, 723, 698, 678, 652 cm^{-1}; MS (ESI +ve) *m/z* 752 ($[M - 2HCl + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{40}H_{55}N_{12}O_3$ 751.4520, found 751.4544 ($[M - 2HCl + H]^+$).

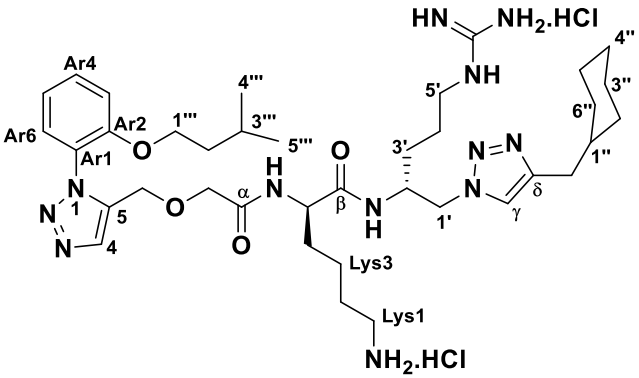
(*R*)-6-Amino-*N*-((*R*)-1-(4-cyclohexyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(2-(isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)acetamido)hexanamide dihydrochloride (47)



Following **General Procedure IV**, azide **68** (0.07 g, 0.07 mmol), cyclohexylacetylene (0.02 g, 0.21 mmol), $CuSO_4 \cdot 5H_2O$ (0.003 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.02 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **159** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 6:94). Following **General Procedure VII**, the intermediate **159** (0.06 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.11 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **47** (0.034 g, 61% over two steps) as a light brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +57.1 (*c* 0.0052, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (s, 2H, H4/Hγ), 7.57 (ddd, *J* = 8.0, 8.0, 3.0 Hz, 1H, Ar4), 7.45-7.42 (m, 1H, Ar6), 7.27-7.25 (m, 1H, Ar5), 7.16-7.11 (m, 1H, Ar3), 4.66 (m, 3H, C5-C$\underline{H}_2$/H1'), 4.46 (apparent t, *J* = 10.5 Hz, 1H, H1'), 4.40-4.31 (m, 1H, Lys5), 4.19-4.15 (m, 1H, H2'), 4.06 (t, *J* = 6.0 Hz, 2H, H1'''), 3.88 (s, 2H, OC$\underline{H}_A\underline{H}_B$), 3.20-3.17 (m, 2H, H5'), 2.93-2.90 (m, 2H, Lys1), 2.84-2.74 (m, 1H, H1''), 2.08-1.96 (m, 2H, H3'), 1.86-1.79 (m, 2H, Lys4), 1.78-1.55 (m, 9H, Lys2/H4'/H2''/H3''/H4''/H5''/H6''), 1.54-1.37 (m, 8H, H2'''/H3'''/H2''/H3''/H4''/H5''/H6''), 1.35-1.18 (m, 2H, Lys3), 0.82 (d, *J* = 6.2 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 172.6 (βC=O), 170.2 (αC=O), 157.2 (C=N), 153.5 (Ar2), 132.06 (C5), 132.02 (Cδ), 128.4 (C4), 124.8 (Ar4), 120.66 (Ar6), 120.61 (Cγ), 115.7 (Ar1), 113.3 (Ar5),

111.8 (Ar3), 68.8 (O<u>C</u>H_AH_B), 67.2 (C1'''), 61.5 (C5-<u>C</u>H_2), 54.2 (C1'), 52.8 (Lys5), 49.1 (C2'), 40.5 (C5'), 39.1 (Lys1), 37.3 (C2'''), 34.5 (C1''), 32.3 (C2''/C6''), 31.3 (Lys4), 28.5 (C3'), 26.6 (Lys2), 25.6 (C4''), 25.4 (C3''/C5''), 24.8 (C3'''), 24.7 (C4'), 22.3 (C4'''/C5'''), 21.3 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3396, 3372, 3358, 3285, 3274, 3263, 3212, 3198, 3067, 2931, 2865, 2162, 2126, 1978, 1965, 1659, 1533, 1509, 1464, 1386, 1368, 1345, 1288, 1247, 1229, 1161, 1129, 1052, 979, 759, 672, 618, 572 cm^{-1}; MS (ESI +ve) *m/z* 723 ($[M - 2HCl + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{36}H_{59}N_{12}O_4$ 723.4782, found 723.4810 ($[M - 2HCl + H]^+$).

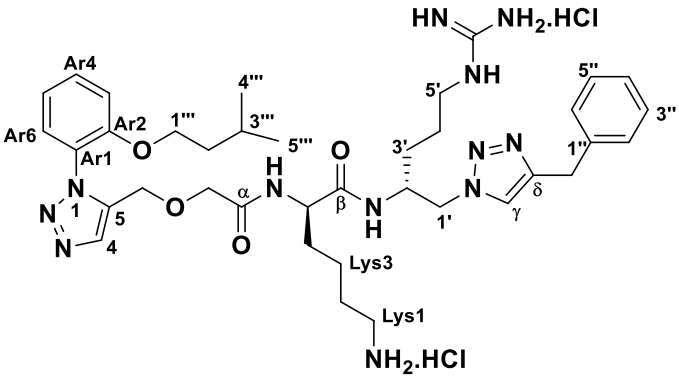
(*R*)-6-Amino-*N*-((*R*)-1-(4-(cyclohexylmethyl)-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(2-(isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)acetamido) hexanamide dihydrochloride (48)



Following **General Procedure IV**, azide **68** (0.07 g, 0.07 mmol), 3-cyclohexyl-1-propyne (0.03 g, 0.21 mmol), $CuSO_4 \cdot 5H_2O$ (0.003 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.02 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **160** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **160** (0.06 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.10 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **48** (0.037 g, 83% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +62.3 (*c* 0.0052, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.19 (s, 1H, H4), 7.97 (s, 1H, Hγ), 7.61-7.56 (m, 1H, Ar4), 7.46-7.44 (m, 1H, Ar6), 7.27-

7.25 (m, 1H, Ar5), 7.15-7.11 (m, 1H, Ar3), 4.72-4.44 (m, 4H, C5-C$\underline{H}_2$/H1'), 4.42-4.26 (m, 1H, Lys5), 4.22-4.12 (m, 1H, H2'), 4.06 (t, *J* = 7.6 Hz, 2H, H1'''), 3.91 (s, 2H, OC$\underline{H}_A\underline{H}_B$), 3.24-3.16 (m, 2H, H5'), 2.97-2.87 (m, 2H, Lys1), 2.70-2.62 (m, 2H, δC-C$\underline{H}_2$), 1.78-1.54 (m, 14H, H3'/Lys2/Lys4/H4'/H1''/H2''/H3''/H4''/H5''/H6''), 1.58-1.44 (m, 3H, H2''/H4''/H6''), 1.38-1.10 (m, 5H, H2'''/H3'''/Lys3), 1.06-0.91 (m, 2H, H3''/H5''), 0.82 (d, *J* = 7.7 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 172.7 (βC=O), 170.2 (αC=O), 157.2 (C=N), 153.6 (Ar2), 132.0 (C5), 128.1 (Cδ), 125.9 (C4), 124.5 (Ar4), 120.67 (Ar6), 120.64 (Cγ), 115.5 (Ar1, Observed by gHMBC), 113.39 (Ar5), 113.36 (Ar3), 68.9 (O$\underline{C}H_AH_B$), 67.3 (C1'''), 61.4 (C5-$\underline{C}H_2$), 55.0 (C1'), 53.1 (Lys5), 52.9 (C2'), 49.3 (δC-$\underline{C}H_2$), 40.5 (C5'), 39.1 (Lys1), 37.6 (C2'''), 37.3 (C1''), 32.4 (C2''), 32.3 (C6''), 31.2 (Lys4), 28.3 (C3'), 26.5 (Lys2), 25.8 (C4''), 25.7 (C3''), 25.6 (C5''), 24.8 (C3'''), 24.7 (C4'), 22.4 (C4'''/C5'''), 21.4 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3381, 3359, 3352, 3333, 3284, 3270, 3260, 3250, 3202, 3074, 3063, 2926, 2869, 2176, 2123, 2061, 1999, 1659, 1529, 1509, 1465, 1450, 1386, 1369, 1288, 1246, 1229, 1137, 1056, 998, 981, 849, 756, 667, 638, 619, 588, 572 cm^{-1}; MS (ESI +ve) *m/z* 737 ([M – 2HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{37}H_{61}N_{12}O_4$ 737.4939, found 737.4940 ([M – 2HCl + H]$^+$).

(*R*)-6-Amino-*N*-((*R*)-1-(4-benzyl-1*H*-1,2,3-triazol-1-yl)-5-guanidinopentan-2-yl)-2-(2-((1-(2-(isopentyloxy)phenyl)-1*H*-1,2,3-triazol-5-yl)methoxy)acetamido)hexanamide dihydrochloride (49)



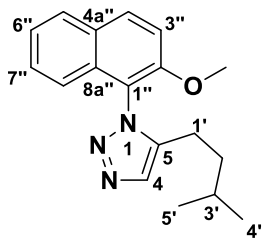
Following **General Procedure IV**, azide **68** (0.08 g, 0.08 mmol), 3-phenyl-1-propyne (0.03 g, 0.24 mmol), $CuSO_4 \cdot 5H_2O$ (0.004 g, 0.01 mmol) and sodium ascorbate (0.006 g, 0.03 mmol) were stirred in *t*-BuOH (2 mL) and H_2O (0.5 mL) for 16 h to give the intermediate **161** as an off-white gum after flash chromatography over SiO_2 gel ($MeOH/CH_2Cl_2$ – 0:100 → 5:95). Following **General Procedure VII**, the intermediate **161** (0.06 g, 0.05 mmol) was dissolved in CH_2Cl_2 (2 mL), treated with H_2O (0.02 g, 1.10 mmol) and CF_3COOH (1 mL) followed by work-up with ethereal HCl (3 mL) to give the amine salt **49** (0.033 g, 51% over two steps) as a pale brown solid that rapidly transitioned to a sticky gum. $[\alpha]_D^{23}$ +68.1 (*c* 0.0048, MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ 8.15 (s, 1H, H4), 8.05 (s, 1H, Hγ), 7.58 (t, *J* = 7.7 Hz, 1H, Ar4), 7.45 (d, *J* = 7.6 Hz, 1H, Ar6), 7.33-7.20 (m, 6H, Ar5/H2''/H3''/H4''/ H5''/H6''), 7.13 (d, *J* = 7.5 Hz, 1H, Ar3), 4.73-4.54 (m, 3H, C5-C$\underline{H}_2$/H1'), 4.56-4.4.45 (m, 1H, H1'), 4.40-4.26 (m, 1H, Lys5), 4.22-4.10 (m, 3H, H2'/OC$\underline{H}_A\underline{H}_B$), 4.10-4.02 (m, 2H, H5'), 3.91 (t, *J* = 5.9 Hz, 2H, H1'''), 3.25-3.11 (m, 2H, Lys1), 2.98-2.81 (m, 2H, δC-C$\underline{H}_2$), 1.73-1.34 (m, 11H, H3'/Lys2/Lys4/ H4'/H2'''/H3'''), 1.31-1.16 (m, 2H, Lys3), 0.82 (d, *J* = 6.1 Hz, 6H, H4'''/H5'''); ^{13}C NMR (101 MHz, CD_3OD) δ 172.7 (βC=O), 170.1 (αC=O), 157.2 (C=N), 153.5 (Ar2), 137.09 (C5), 137.03 (Cδ), 132.2 (C1''), 128.67 (C2''), 128.65 (C6''), 128.5 (C3''), 128.4 (C5''), 128.1 (C4), 126.9 (Ar4), 126.8 (C4''), 126.7 (Ar6), 125.8 (Cγ), 124.3 (Ar1), 120.6 (Ar5), 113.3 (Ar3),

68.9 (O<u>C</u>H$_A$H$_B$), 67.3 (C1'''), 61.4 (C5-<u>C</u>H$_2$), 54.9 (C1'), 52.8 (Lys5), 49.2 (C2'), 40.5 (C5'), 39.1 (Lys1), 37.3 (C2'''), 31.2 (δC-<u>C</u>H$_2$), 29.9 (Lys4), 28.3 (C3'), 26.5 (Lys2), 24.8 (C3'''), 24.7 (C4'), 22.5 (C4'''/C5'''), 21.4 (Lys3); IR (neat) $\bar{\nu}_{max}$ 3358, 3273, 3195, 3068, 2955, 2871, 2165, 2101, 2063, 1658, 1533, 1509, 1464, 1386, 1368, 1288, 1245, 1227, 1164, 1137, 1050, 999, 979, 875, 850, 757, 724, 699667, 621, 590 cm^{-1}; MS (ESI +ve) *m/z* 731 ([M – 2HCl + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{37}H_{55}N_{12}O_4$ 731.4469, found 731.4444 ([M – 2HCl + H]$^+$).

6.4 – Experimental-Click chemistry

6.4.1 – Magnesium promoted click reactions

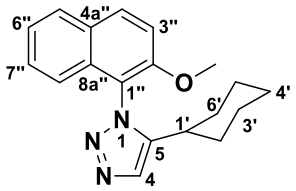
5-Isopentyl-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole (83)



This compound was prepared according to the **General procedure VI**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 5-methyl-1-hexyne (0.03 g, 0.27 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 3 h to give **83** (0.06 g, 81%) as a pale brown waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, *J* = 9.1 Hz, 1H, H8''), 7.85 (dd, *J* = 7.4, 2.0 Hz, 1H, H5''), 7.71 (s, 1H, H4), 7.45-7.37 (m, 3H, H6'', H7'' and H4''), 6.99-6.96 (m, 1H, H3''), 3.87 (s, 3H, OMe), 2.45-2.34 (m, 2H, H1'), 1.47-1.35 (m, 3H, H2' and H3'), 0.73 (d, *J* = 7.9 Hz, 3H, H5'), 0.72 (d, *J* = 7.9 Hz, 3H, H4'); ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.7 (C2''), 140.7 (C5), 132.0 (C8a''), 131.6 (C4), 131.4 (C4a''), 128.7 (C4''), 128.3 (C5''), 127.9 (C7''), 124.6 (C8''), 121.2 (C6''), 117.9 (C3''), 113.1 (C1''), 56.5 (OMe), 36.7 (C2'), 27.2 (C1'), 22.08 (C5'), 22.07 (C4'), 20.8 (C3'); IR (neat) ν_{max} 2954, 2868, 1630, 1599, 1507, 1457, 1275,

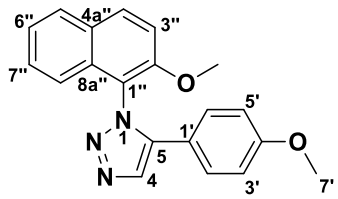
1255, 1064, 1040, 809, 748 cm^{-1}; MS (ESI +ve) *m/z* 296 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{18}H_{22}N_3O$ 296.1763, found 296.1750 ($[M + H]^+$).

5-Cyclohexyl-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole (100)



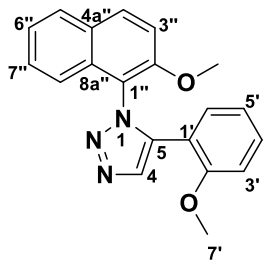
This compound was prepared according to the **General procedure VI**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with ethynylcyclohexane (0.03 g, 0.27 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 5 h to give **100** (0.06 g, 78%) as a cream white solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 30:70 → 100:0). M.P: 104-106 °C; TLC (EtOAc/*n*-hexane 1:4): R_f = 0.1; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.03 (d, *J* = 9.1 Hz, 1H, H8''), 7.87-7.85 (m, 1H, H5''), 7.71 (s, 1H, H4), 7.44-7.38 (m, 3H, H6'', H7'' and H4''), 6.95 (dd, *J* = 8.2, 1.2 Hz, 1H, H3''), 3.87 (s, 3H, OMe), 2.31-2.24 (m, 1H, H1'), 1.91-1.88 (m, 1H, H5'), 1.74-1.70 (m, 1H, H4'), 1.60-1.56 (m, 3H, H6', H2' and H3'), 1.43-1.40 (m, 1H, H5'), 1.32-1.29 (m, 1H, H4'), 1.19-1.10 (m, 2H, H6' and H3'), 1.01-0.97 (m, 1H, H2'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.7 (C2''), 145.7 (C5), 132.0 (C8a''), 131.8 (C4), 130.1 (C4''), 128.7 (C4a''), 128.3 (C5''), 127.9 (C7''), 124.6 (C8''), 121.3 (C6''), 118.1 (C3''), 113.1 (C1''), 56.6 (OMe), 33.3 (C6'), 32.9 (C2'), 32.4 (C1'), 25.9 (C4'), 25.8 (C5'), 25.6 (C3'); IR (neat) ν_{max} 2927, 2852, 1628, 1599, 1510, 1482, 1276, 1245, 1151, 1067, 818, 751 cm^{-1}; MS (ESI +ve) *m/z* 308 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{22}N_3O$ 308.1763, found 308.1750 ($[M + H]^+$).

1-(2-Methoxynaphthalen-1-yl)-5-(4-methoxyphenyl)-1*H*-1,2,3-triazole (101)



This compound was prepared according to the **General procedure VI**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 1-ethynyl-4-methoxybenzene (0.04 g, 0.27 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 4 h to give **101** (0.072 g, 86%) as a waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (s, 1H, H4), 7.96 (d, *J* = 9.6 Hz, 1H, H8''), 7.81 (d, *J* = 8.0 Hz, 1H, H5''), 7.43-7.35 (m, 2H, H6'' and H7''), 7.28 (d, *J* = 9.1 Hz, 1H, H4''), 7.16 (d, *J* = 8.3 Hz, 1H, H3''), 7.09-7.06 (m, 2H, H2' and H6'), 6.69-6.66 (m, 2H, H3' and H5'), 3.69 (s, 3H, OMe), 3.66 (s, 3H, H7'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.0 (C4'), 152.6 (C2''), 140.2 (C8a''), 132.0 (C4), 131.65 (C4''), 131.63 (C4a''), 128.7 (C2' and C6'), 128.4 (C5''), 127.9 (C7''), 127.8 (C8''), 124.6 (C6''), 121.3 (C3''), 119.2 (C5), 118.6 (C1'), 114.0 (C3' and C5'), 113.2 (C1''), 56.5 (OMe), 55.1 (C7'); IR (neat) ν_{max} 2940, 2839, 1616, 1558, 1507, 1495, 1457, 1276, 1249, 1179, 1067, 1020, 810, 750 cm^{-1}. MS (ESI +ve) *m/z* 332 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{20}H_{18}N_3O_2$ 332.1399, found 332.1386 ([M + H]$^+$).

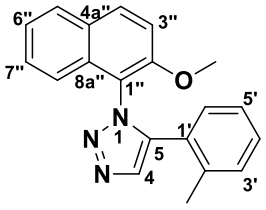
1-(2-Methoxynaphthalen-1-yl)-5-(2-methoxyphenyl)-1*H*-1,2,3-triazole (102)



This compound was prepared according to the **General procedure VI**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 1-ethynyl-2-methoxybenzene (0.04 g, 0.27 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 4 h to give **102** (0.074 g, 89%) as an off-white waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). TLC (EtOAc/*n*-hexane

2:3): R_f = 0.1; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (s, 1H, H4), 7.89 (d, *J* = 9.1 Hz, 1H, H6'), 7.79 (d, *J* = 8.1 Hz, 1H, H8''), 7.46-7.43 (m, 1H, H5''), 7.39-7.35 (m, 2H, H6'' and H7''), 7.21-7.17 (m, 2H, H4' and H5'), 7.06 (dd, *J* = 8.0, 1.7 Hz, 1H, H4''), 6.75 (d, *J* = 7.5 Hz, 1H, H3'), 6.70 (d, *J* = 8.0 Hz, 1H, H3''), 3.59 (s, 3H, OMe), 3.26 (s, 3H, H7'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.6 (C2'), 151.9 (C2''), 137.2 (C8a''), 133.7 (C4), 131.48 (C6'), 131.46 (C4''), 130.5 (C4'), 130.3 (C4a''), 128.7 (C5''), 127.8 (C7''), 127.6 (C8''), 124.3 (C6''), 122.3 (C5'), 120.1 (C5), 119.3 (C3''), 116.2 (C1'), 113.0 (C1''), 110.7 (C3'), 56.3 (C7'), 54.7 (OMe); IR (neat) ν_{max} 2938, 2840, 1507, 1486, 1465, 1264, 1150, 1104, 1067, 1020, 806, 749 cm^{-1}. MS (ESI +ve) *m/z* 332 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{20}H_{18}N_3O_2$ 332.1399, found 332.1388 ([M + H]$^+$).

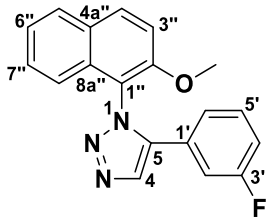
1-(2-Methoxynaphthalen-1-yl)-5-(*o*-tolyl)-1*H*-1,2,3-triazole (103)



This compound was prepared according to the **General procedure VI**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 1-ethynyl-2-methylbenzene (0.03 g, 0.27 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (3 mL) at 50 °C for 3 h to give **103** (0.06 g, 76%) as a cream waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 30:70 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (s, 1H, H4), 7.90 (d, *J* = 9.1 Hz, 1H, H8''), 7.80 (d, *J* = 8.2 Hz, 1H, H5''), 7.50-7.46 (m, 1H, H6''), 7.40-7.37 (m, 1H, H7''), 7.33 (dd, *J* = 8.8, 0.6 Hz, 1H, H4''), 7.18 (d, *J* = 8.8 Hz, 1H, H3''), 7.15-7.11 (m, 2H, H3' and H6'), 6.87-6.86 (m, 2H, H4' and H5'), 3.67 (s, 3H, OMe), 2.27 (s, 3H, Me); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.1 (C2''), 139.5 (C8a''), 137.1 (C2'), 133.5 (C4), 131.8 (C4a''), 131.6 (C4''), 130.1 (C1'), 129.5 (C3'), 129.0 (C8''), 128.6 (C5''), 128.2 (C7''), 127.9 (C4'), 126.5 (C5'), 125.3 (C6''),

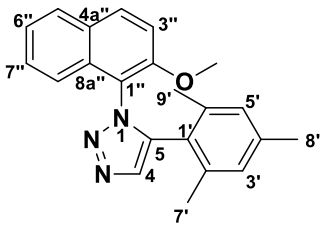
124.5 (C3"), 121.5 (C6'), 118.2 (C5), 112.7 (C1"), 56.1 (OMe), 20.1 (Me); IR (neat) ν_{max} 3140, 2929, 2847, 1599, 1510, 1274, 1109, 1244, 1058, 1021, 815, 760 cm^{-1}. MS (ESI +ve) *m/z* 316 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{20}H_{18}N_3O$ 316.1450, found 316.1448 ($[M + H]^+$).

5-(3-Fluorophenyl)-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole (104)



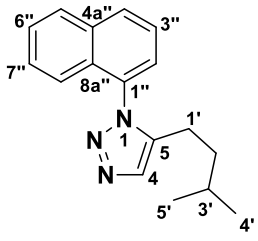
This compound was prepared according to the **General procedure VI**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 1-ethynyl-3-fluorobenzene (0.03 g, 0.27 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 5 h to give **104** (0.069 g, 86%) as a light-yellow solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). M.P: 148-150 °C. TLC (EtOAc/*n*-hexane 1:4): R_f = 0.3; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.05 (s, 1H, H4), 8.00 (d, *J* = 9.1 Hz, 1H, H8"), 7.84 (d, *J* = 8.1 Hz, 1H, H5"), 7.46-7.43 (m, 1H, H6"), 7.41-7.37 (m, 1H, H7"), 7.30 (d, *J* = 9.1 Hz, 1H, H4"), 7.16-7.11 (m, 2H, H3" and H2'), 6.95-6.86 (m, 3H, H4', H5' and H6'), 3.72 (s, 3H, OMe); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.6 (d, $^1J_{C\text{-}F}$ = 198.3 Hz, C3'), 152.7 (C2"), 139.3 (C8a"), 132.56 (C4a"), 132.52 (C4), 131.6 (C8"), 130.4 (d, $^2J_{C\text{-}F}$ = 7.0, C1'), 129.1 (d, $^3J_{C\text{-}F}$ = 10.1, C5'), 128.79 (C4"), 128.77 (C5"), 128.2 (C3"), 124.8 (C6"), 123.3 (C7"), 121.2 (C6'), 118.3 (C5), 116.1 (d, $^4J_{C\text{-}F}$ = 16.9 Hz, C2'), 114.5 (d, $^5J_{C\text{-}F}$ = 18.6 Hz, C4'), 113.2 (C1"), 56.6 (OMe); IR (neat) ν_{max} 2937, 2842, 1558, 1507, 1457, 1273, 1149, 1110, 1069, 862, 807, 798, 690 cm^{-1}. MS (ESI +ve) *m/z* 320 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{15}FN_3O$ 320.1199, found 320.1205 ($[M + H]^+$).

5-Mesityl-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole (105)



This compound was prepared according to the **General procedure VI**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 2-ethynyl-1,3,5-trimethylbenzene (0.04 g, 0.27 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 6 h to give **105** (0.045 g, 52%) as a brown waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). TLC (EtOAc/*n*-hexane 2:3): R_f = 0.1; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (d, *J* = 7.3 Hz, 1H, H8''), 7.83 (s, 1H, H4), 7.77 (d, *J* = 7.3 Hz, 1H, H5''), 7.50-7.44 (m, 2H, H6'' and H4''), 7.38-7.35 (m, 1H, H7''), 7.12-7.10 (m, 1H, H3''), 6.89 (s, 1H, H5'), 6.57 (s, 1H, H3'), 3.57 (s, 3H, OMe), 2.25 (s, 3H, H9'), 2.18 (s, 3H, H7'), 1.57 (s, 3H, H8'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.6 (C2''), 138.9 (C8a''), 138.8 (C4'), 138.7 (C2'), 137.8 (C6'), 134.4 (C4), 131.8 (C5''), 130.9 (C4a''), 128.9 (C4''), 128.3 (C3'), 128.1 (C5'), 128.0 (C7''), 127.9 (C8''), 124.4 (C6''), 123.3 (C1'), 122.7 (C5), 118.4 (C3''), 112.7 (C1''), 55.6 (OMe), 21.1 (C8'), 20.8 (C9'), 20.7 (C7'); IR (neat) ν_{max} 2924, 2845, 1710, 1630, 1511, 1481, 1357, 1276, 1219, 1066, 1021, 967, 810, 749 cm^{-1}. MS (ESI +ve) *m/z* 344 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{22}H_{22}N_3O$ 344.1763, found 344.1758 ([M + H]$^+$).

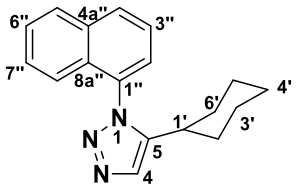
5-Isopentyl-1-(naphthalen-1-yl)-1*H*-1,2,3-triazole (114)



This compound was prepared according to the **General procedure VI**, 1-azidonaphthalene **99** (0.05 g, 0.29 mmol) was treated with 5-methyl-1-hexyne (0.03 g, 0.32 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 4 h to give **114** (0.068 g, 88%) as a brown waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-

hexane - 30:70 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.3 Hz, 1H, H8''), 7.95 (d, *J* = 8.2 Hz, 1H, H5''), 7.70 (s, 1H, H4), 7.61-7.54 (m, 2H, H6'' and H7''), 7.50-7.47 (m, 2H, H3'' and H4''), 7.16 (dd, *J* = 8.5, 0.8 Hz, 1H, H2''), 2.45-2.42 (m, 2H, H1'), 1.46-1.36 (m, 3H, H2' and H3'), 0.72 (d, *J* = 6.2 Hz, 6H, H4' and H5'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 140.3 (C5), 134.2 (C8a''), 132.7 (C4a''), 131.8 (C4), 130.7 (C8''), 130.0 (C5''), 128.4 (C4''), 128.0 (C6''), 127.2 (C7''), 125.2 (C3''), 125.1 (C1''), 122.4 (C2''), 37.2 (C2'), 27.4 (C3'), 22.2 (C4' and C5'), 21.3 (C1'); IR (neat) ν_{max} 2955, 2868, 1598, 1468, 1430, 1247, 1077, 960, 802, 773, 661 cm^{-1}. MS (ESI +ve) *m/z* 266 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{17}H_{20}N_3$ 266.1657, found 266.1668 ($[M + H]^+$).

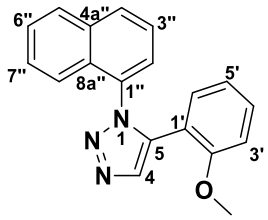
5-Cyclohexyl-1-(naphthalen-1-yl)-1*H*-1,2,3-triazole (115)



This compound was prepared according to the **General procedure VI**, 1-azidonaphthalene **99** (0.05 g, 0.29 mmol) was treated with ethynylcyclohexane (0.03 g, 0.32 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 4 h to give **115** (0.071 g, 88%) as a brown gummy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.3 Hz, 1H, H8''), 7.95 (d, *J* = 8.2 Hz, 1H, H5''), 7.70 (s, 1H, H4), 7.61-7.54 (m, 2H, H6'' and H7''), 7.50-7.46 (m, 2H, H3'' and H4''), 7.12 (dd, *J* = 8.5, 0.8 Hz, 1H, H2''), 2.40-2.34 (m, 1H, H1'), 1.77-1.70 (m, 2H, H2' and H6'), 1.66-1.55 (m, 3H, H3', H4' and H5'), 1.41-1.33 (m, 2H, H3' and H5'), 1.20-1.12 (m, 1H, H4'), 1.08-1.00 (m, 2H, H2' and H6'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 145.3 (C5), 134.2 (C8a''), 132.9 (C4a''), 130.8 (C4), 130.5 (C8''), 130.3 (C5''), 128.3 (C4''), 128.0 (C6''), 127.2 (C7''), 125.2 (C3''), 125.1 (C1''), 122.4 (C2''), 33.4 (C1'), 25.9 (C2', C4', and C6'), 25.6 (C3' and C5'); IR (neat) ν_{max} 2929, 2852, 1710, 1597,

1448, 1238, 1084, 1005, 960, 802, 772 cm^{-1}. MS (ESI +ve) *m/z* 278 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{18}H_{20}N_3$ 278.1657, found 278.1659 ($[M + H]^+$).

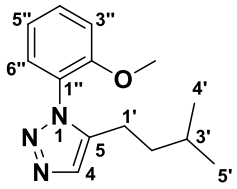
5-(2-Methoxyphenyl)-1-(naphthalen-1-yl)-1*H*-1,2,3-triazole (116)



This compound was prepared according to the **General procedure VI**, 1-azidonaphthalene **99** (0.05 g, 0.29 mmol) was treated with 1-ethynyl-2-methoxybenzene (0.04 g, 0.32 mmol) and EtMgCl (0.03 g, 0.3 mmol; 2 M in Et_2O) in dry THF (2 mL) at 50 °C for 12 h to give **116** (0.009 g, 10%) as a brown waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 30:70 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.1; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.99 (s, 1H, H4), 7.91-7.88 (m, 2H, H8" and H6'), 7.62-7.60 (m, 1H, H5"), 7.54-7.48 (m, 2H, H6" and H7"), 7.39 (dd, *J* = 7.4, 1.0 Hz, 1H, H3"), 7.26-7.21 (m, 2H, H4" and H5'), 7.14 (dd, *J* = 7.4, 1.7 Hz, 1H, H2"), 6.83 (dt, *J* = 9.4, 1.2 Hz, 1H, H4'), 6.69 (dd, *J* = 8.4, 1.0 Hz, 1H, H3'), 3.16 (s, 3H, OMe); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.6 (C2'), 136.8 (C8a"), 134.2 (C4a"), 134.1 (C1'), 133.8 (C4), 131.1 (C5"), 130.9 (C2"), 130.0 (C8"), 129.6 (C4"), 128.0 (C6'), 127.5 (C7"), 126.8 (C6"), 124.8 (C3"), 124.1 (C1"), 123.5 (C5), 120.6 (C4'), 115.8 (C5'), 111.0 (C3'), 54.7 (OMe); IR (neat) ν_{max} 3051, 2928, 1597, 1481, 1464, 1270, 1103, 1050, 1023, 955, 802, 754 cm^{-1}. MS (ESI +ve) *m/z* 302 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{16}N_3O$ 302.1293, found 302.1305 ($[M + H]^+$).

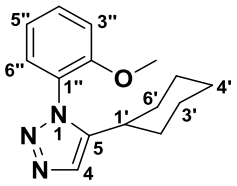
6.4.2 – Ruthenium catalysed click reactions

5-Isopentyl-1-(2-methoxyphenyl)-1*H*-1,2,3-triazole (119)



This compound was prepared according to the **General procedure V**, 1-azido-2-methoxybenzene **118** (0.02 g, 0.13 mmol) was treated with 5-methyl-1-hexyne (0.014 g, 0.15 mmol) and Cp*RuCl(PPh$_3$)$_2$ (0.01 g, 0.01 mmol) in 1,4-dioxane (2 mL) at 70 °C for 12 h to give **119** (0.028 g, 87%) as a brown oil after purification by column chromatography over SiO$_2$ gel (EtOAc/*n*-hexane - 30:70 → 100:0). TLC (EtOAc/*n*-hexane 2:3): R_f = 0.5; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.55 (s, 1H, H4), 7.50-7.47 (m, 1H, H4''), 7.32 (dd, *J* = 7.9, 1.7 Hz, 1H, H6''), 7.11-7.06 (m, 2H, H3''and H5''), 3.78 (s, 3H, OMe), 2.47 (t, *J* = 7.9 Hz, 2H, H1'), 1.56-1.41 (m, 3H, H2' and H3'), 0.82 (d, *J* = 8.0 Hz, 6H, H4' and H5'); ^{13}C NMR (126 MHz, CDCl$_3$) δ 154.2 (C2''), 140.0 (C5), 131.5 (C4''), 131.3 (C4), 128.7 (C6''), 125.1 (C1''), 120.9 (C5''), 112.1 (C3''), 55.7 (OMe), 36.9 (C2'), 27.4 (C3'), 22.1 (C4' and C5'), 21.0 (C1'); IR (neat) ν_{max} 2954, 2868, 1602, 1506, 1473, 1285, 1251, 1233, 1122, 1043, 1021, 750 cm^{-1}. MS (ESI +ve) *m/z* 246 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{14}H_{20}N_3O$ 246.1606, found 246.1618 ([M + H]$^+$).

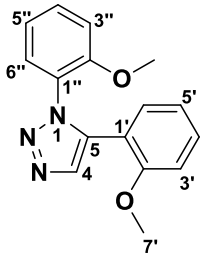
5-Cyclohexyl-1-(2-methoxyphenyl)-1*H*-1,2,3-triazole (120)



This compound was prepared according to the **General procedure V**, 1-azido-2-methoxybenzene **118** (0.05 g, 0.33 mmol) was treated with ethynyl cyclohexane (0.04 g, 0.37 mmol) and Cp*RuCl(PPh$_3$)$_2$ (0.03 g, 0.03 mmol) in 1,4-dioxane (2 mL) at 70 °C for 12 h to give **120** (0.066 g, 78%) as a yellow waxy solid after purification by column chromatography over SiO$_2$ gel (EtOAc/*n*-hexane - 20:80 → 100:0). TLC (EtOAc/*n*-hexane 2:3): R_f = 0.5; ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.56 (s, 1H, H4), 7.52-7.48 (m, 1H, H4''), 7.32 (dd, *J* = 7.6, 1.3 Hz, 1H, H6''), 7.11-7.06 (m, 2H,

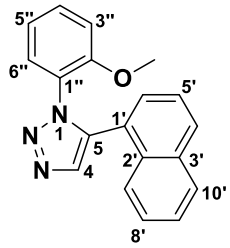
H5'' and H3''), 3.77 (s, 3H, OMe), 2.44-2.38 (m, 1H, H1'), 1.89-1.71 (m, 2H, H2' and H6'), 1.67-1.64 (m, 3H, H3', H4' and H5'), 1.38-1.31 (m, 2H, H3' and H5'), 1.27-1.23 (m, 1H, H4'), 1.22-1.16 (m, 2H, H6' and H2'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 154.3 (C2''), 144.8 (C5), 131.4 (C4''), 130.0 (C4), 128.8 (C6''), 125.3 (C1''), 120.9 (C5''), 112.2 (C3''), 55.8 (OMe), 33.4 (C1'), 26.0 (C2', C4' and C6'), 25.6 (C3' and C5'); IR (neat) ν_{max} 2928, 2850, 1506, 1457, 1280, 1233, 1046, 1020, 994, 974, 838, 770, 671 cm^{-1}. MS (ESI +ve) *m/z* 258 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{15}H_{20}N_3O$ 258.1606, found 258.1602 ($[M + H]^+$).

1,5-Bis(2-methoxyphenyl)-1*H*-1,2,3-triazole (121)



This compound was prepared according to the **General procedure V**, 1-azido-2-methoxybenzene **118** (0.05 g, 0.33 mmol) was treated with 1-ethynyl-2-methoxybenzene (0.05 g, 0.37 mmol) and Cp*RuCl(PPh_3)$_2$ (0.03 g, 0.03 mmol) in 1,4-dioxane (2 mL) at 70 °C for 36 h to give **121** (0.054 g, 58%) as a pale yellow waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 30:70 → 100:0). TLC (EtOAc/*n*-hexane 2:3): R_f = 0.3; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (s, 1H, H4), 7.41 (dd, *J* = 7.8, 1.7 Hz, 1H, H3'), 7.38-7.34 (m, 1H, H4''), 7.30-7.26 (m, 1H, H5'), 7.10 (dd, *J* = 8.0, 1.7 Hz, 1H, H6''), 7.02 (ddd, *J* = 7.6, 1.2 Hz, 1H, H4'), 6.88-6.85 (m, 2H, H5'' and H6'), 6.83 (dd, *J* = 8.0, 1.0 Hz, 1H, H3''), 3.56 (s, 3H, OMe), 3.46 (s, 3H, H7'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.5 (C2'), 153.4 (C2''), 136.1 (C5), 133.6 (C4), 130.7 (C4''), 130.5 (C6'), 130.3 (C4'), 128.0 (C6''), 126.4 (C1''), 120.5 (C5''), 120.3 (C5'), 116.7 (C1'), 112.0 (C3''), 110.9 (C3'), 55.4 (C7'), 55.0 (OMe); IR (neat) ν_{max} 2932, 2838, 1602, 1506, 1471, 1465, 1436, 1283, 1246, 1108, 1022, 989, 753 cm^{-1}. MS (ESI +ve) *m/z* 282 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{16}H_{16}N_3O_2$ 282.1243, found 282.1244 ($[M + H]^+$).

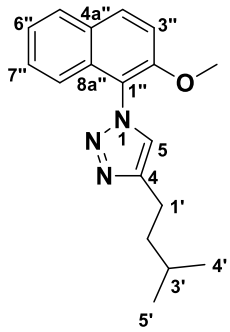
1-(2-Methoxyphenyl)-5-(naphthalen-1-yl)-1*H*-1,2,3-triazole (122)



This compound was prepared according to the **General procedure V**, 1-azido-2-methoxybenzene **118** (0.05 g, 0.33 mmol) was treated with 1-ethynylnaphthalene (0.056 g, 0.37 mmol) and Cp*RuCl(PPh_3)$_2$ (0.03 g, 0.03 mmol) in 1,4-dioxane (2 mL) at 70 °C for 48 h to give **122** (0.007 g, 7%) as a pale brown waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 30:70 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.96 (s, 1H, H4), 7.88-7.81 (m, 3H, H4', H7' and H10'), 7.50-7.44 (m, 3H, H5', H6' and H9'), 7.34-7.28 (m, 2H, H4" and H8'), 7.16 (dd, *J* = 7.1, 1.1 Hz, 1H, H6"), 6.96 (td, *J* = 7.7, 1.1 Hz, 1H, H5"), 6.72 (dd, *J* = 8.3, 0.9 Hz, 1H, H3"), 3.14 (s, 3H, OMe); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.2 (C2"), 137.4 (C5), 134.2 (C1'), 133.3 (C4), 131.6 (C3'), 131.0 (C4"), 129.4 (C6"), 128.2 (C2'), 128.1 (C7'), 127.6 (C10'), 126.7 (C8'), 126.2 (C9'), 125.2 (C1"), 125.1 (C4'), 124.9 (C5'), 124.7 (C6'), 120.6 (C5"), 111.8 (C3"), 54.9 (OMe); IR (neat) ν_{max} 2925, 2849,1602, 1506, 1437, 1275, 1248, 1048, 1021, 977, 803, 778, 755 cm^{-1}. MS (ESI +ve) *m/z* 302 ([M + H]$^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{16}N_3O$ 302.1293, found 302.1297 ([M + H]$^+$).

6.4.3 – Copper catalysed click reactions

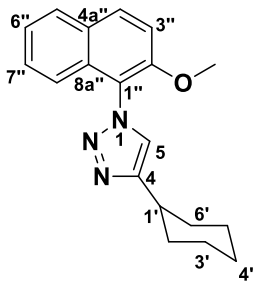
4-Isopentyl-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole (123)



This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 5-methyl-1-hexyne (0.05 g, 0.5 mmol), $CuSO_4.5H_2O$ (0.012 g, 0.05 mmol) and Na. ascorbate (0.02 g, 0.1 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 36 h to give **123** (0.063 g, 85%) as a brown waxy solid after

purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 11.3 Hz, 1H, H8''), 7.83 (d, *J* = 9.5 Hz, 1H, H5''), 7.52 (s, 1H, H5), 7.46-7.35 (m, 3H, H6'', H7'' and H4''), 7.16 (d, *J* = 10.5 Hz 1H, H3''), 3.87 (s, 3H, OMe), 2.91-2.87 (m, 2H, H1'), 1.73-1.69 (m, 3H, H2' and H3'), 0.99 (d, *J* = 7.9, 6H, H4' and H5'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.1 (C2''), 131.6 (C8a''), 131.2 (C4), 128.6 (C4''), 128.2 (C4a''), 127.8 (C5''), 124.5 (C7''), 124.3 (C8''), 122.1 (C6''), 121.4 (C5), 119.7 (C3''), 113.1 (C1''), 56.7 (OMe), 38.4 (C2'), 27.8 (C1'), 23.7 (C3'), 22.4 (C4' and C5'); IR (neat) ν_{max} 2952, 2869, 2352, 1630, 1600, 1507, 1457, 1365, 1275, 1255, 1149, 1110, 1064, 1041, 905, 809, 747 cm^{-1}. MS (ESI +ve) *m/z* 296 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{18}H_{22}N_3O$ 296.1763, found 296.1771 ($[M + H]^+$).

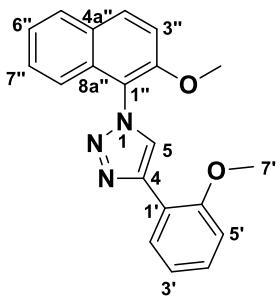
4-Cyclohexyl-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole (124)



This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with ethynylcyclohexane (0.054 g, 0.5 mmol), $CuSO_4.5H_2O$ (0.012 g, 0.05 mmol) and Na. ascorbate (0.02 g, 0.1 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for and 48 h to give **124** (0.062 g, 81%) as a pale yellow waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 30:70 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 9.1 Hz, 1H, H8''), 7.83-7.81 (m, 1H, H5''), 7.48 (s, 1H, H5), 7.44-7.36 (m, 2H, H6'' and H7''), 7.35 (d, *J* = 8.8 Hz, 1H, H4''), 7.14 (dd, *J* = 8.8, 0.7 Hz, 1H, H3''), 3.86 (s, 3H, OMe), 2.93 (tt, *J* = 11.3, 3.5 Hz, 1H, H1'), 2.24-2.20 (m, 2H, H2' and H6'), 1.86 (m, 2H, H3' and H5'), 1.77-1.74 (m, 1H, H4'), 1.58-1.42 (m, 4H, H2', H3', H5' and H6'), 1.35-1.25 (m, 1H, H4'); ^{13}C NMR (126

MHz, $CDCl_3$) δ 153.1 (C2''), 152.1 (C4), 131.6 (C8a''), 131.3 (C4''), 128.6 (C4a''), 128.2 (C5''), 127.7 (C7''), 124.5 (C8''), 123.1 (C5), 121.5 (C6''), 119.8 (C3''), 113.1 (C1''), 56.7 (OMe), 35.3 (C1'), 33.0 (C2' and C6'), 26.2 (C4'), 26.1 (C3' and C5'); IR (neat) ν_{max} 2923, 2850, 1739, 1631, 1599, 1487, 1512, 1448, 1364, 1252, 1235, 1069, 1028, 809, 796, 760, 652 cm^{-1}. MS (ESI +ve) *m/z* 308 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{22}N_3O$ 308.1763, found 308.1775 ($[M + H]^+$).

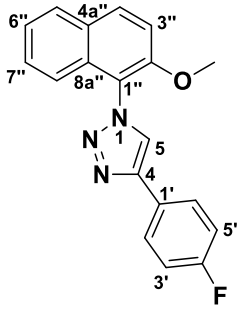
1-(2-Methoxynaphthalen-1-yl)-4-(2-methoxyphenyl)-1*H*-1,2,3-triazole (125)



This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 1-ethynyl-2-methoxybenzene (0.066 g, 0.5 mmol), $CuSO_4.5H_2O$ (0.012 g, 0.05 mmol) and Na. ascorbate (0.02 g, 0.1 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 36 h to give **125** (0.074 g, 89%) as a light brown solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). M.P: 112-114 °C. TLC (EtOAc/*n*-hexane 1:4): R_f = 0.4; ^1H NMR (500 MHz, $CDCl_3$) δ 8.54 (dd, *J* = 7.7, 1.7 Hz, 1H, H2'), 8.33 (s, 1H, H5), 8.02 (d, *J* = 9.1 Hz, 1H, H8''), 7.87-7.85 (m, 1H, H5''), 7.46-7.39 (m, 3H, H6'', H7'' and H4''), 7.36-7.33 (m, 1H, H4'), 7.25-7.23 (m, 1H, H3'), 7.15 (dd, *J* = 7.6, 1.0 Hz, 1H, H5'), 7.00 (dd, *J* = 8.3 Hz, 1H, H3''), 3.90 (s, 3H, OMe), 3.89 (s, 3H, H7'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.6 (C2''), 152.3 (C6'), 142.7 (C4), 131.8 (C8a''), 131.2 (C2'), 128.88 (C4''), 128.84 (C4'), 128.2 (C4a''), 127.8 (C5''), 127.6 (C7''), 126.9 (C5), 124.5 (C8''), 121.4 (C6''), 121.0 (C3'), 119.6 (C1'), 119.3 (C3''), 113.0 (C1''), 110.7 (C5'), 56.7 (C7'), 55.2 (OMe); IR (neat) ν_{max} 2922, 2851, 1601, 1506, 1486, 1465, 1275, 1246, 1076, 1058, 1032, 1020, 795, 751, 742,

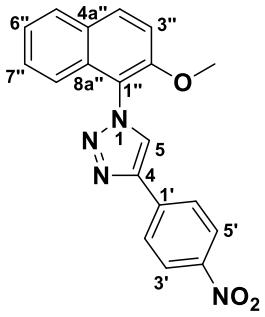
669 cm^{-1}. MS (ESI +ve) *m/z* 332 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{20}H_{18}N_3O_2$ 332.1399, found 332.1394 ($[M + H]^+$).

4-(4-Fluorophenyl)-1-(2-methoxynaphthalen-1-yl)-1*H*-1,2,3-triazole (126)



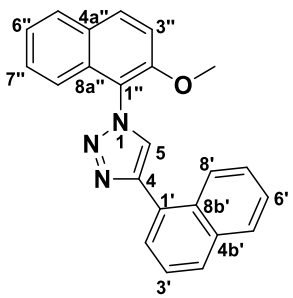
This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 1-ethynyl-4-fluorobenzene (0.06 g, 0.5 mmol), $CuSO_4.5H_2O$ (0.012 g, 0.05 mmol) and Na. ascorbate (0.02 g, 0.1 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 12 h to give **126** (0.074 g, 92%) as an off-white solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 30:70 $\rightarrow$ 100:0). M.P: 109-111 °C. TLC (EtOAc/*n*-hexane 1:4): R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, *J* = 9.1 Hz, 1H, H8''), 7.97 (s, 1H, H5), 7.92 (dd, *J* = 9.1, 2.1 Hz 2H, H2'/H6'), 7.84 (d, *J* = 8.0 Hz, 1H, H5''), 7.46-7.37 (m, 3H, H6'', H7'' and H4''), 7.25 (d, *J* = 8.3 Hz, 1H, H3''), 7.14 (apparent t, *J* = 8.6 Hz, 2H, H3'/H5'), 3.88 (s, 3H, OMe); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.6 (d, J_{C-F} = 198.7 Hz, C4'), 152.1 (C2''), 146.4 (C4), 132.0 (C8a''), 131.0 (C4a''), 128.5 (d, J_{C-F} = 18.1 Hz, C2'/C6'), 127.9 (C4''), 127.58 (C5''), 127.52 (C5), 126.8 (d, J_{C-F} = 2.0 Hz, C1'), 124.7 (C7''), 123.3 (C8''), 121.3 (C6''), 119.2 (C3''), 115.8 (d, J_{C-F} = 86.3 Hz C3'/C5'), 113.0 (C1''), 56.7 (OMe); IR (neat) ν_{max} 2919, 2849, 1714, 1598, 1507, 1482, 1457, 1276, 1258, 1223, 1218, 1146, 1079, 1032, 838, 811, 796, 755, 658 cm^{-1}. MS (ESI +ve) *m/z* 320 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{15}FN_3O$ 320.1199, found 320.1213 ($[M + H]^+$).

1-(2-Methoxynaphthalen-1-yl)-4-(4-nitrophenyl)-1*H*-1,2,3-triazole (127)



This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 1-ethynyl-4-nitrobenzene (0.074 g, 0.5 mmol), $CuSO_4.5H_2O$ (0.012 g, 0.05 mmol) and Na. ascorbate (0.02 g, 0.1 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 16 h to give **127** (0.07 g, 79%) as a yellow solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 30:70 → 100:0). M.P: 118-120 °C. TLC (EtOAc/*n*-hexane 1:4): R_f = 0.3; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (d, *J* = 8.8 Hz, 2H, H3' and H5'), 8.18 (s, 1H, H5), 8.14 (d, *J* = 8.8 Hz, 2H, H2' and H6'), 8.07 (d, *J* = 9.1 Hz, 1H, H8''), 7.89 (d, *J* = 8.0 Hz, 1H, H5''), 7.50-7.45 (m, 2H, H6'' and H7''), 7.43 (d, *J* = 9.1 Hz, 1H, H4''), 7.26 (d, *J* = 9.1 Hz, 1H, H3''), 3.93 (s, 3H, OMe); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.1 (C2''), 147.3 (C4), 145.1 (C8a''), 136.9 (C1'), 132.3 (C4'), 130.8 (C4''), 128.6 (C4a''), 128.0 (C5''), 126.2 (C2'/C6'), 126.1 (C7''), 125.1 (C5), 124.8 (C8''), 124.3 (C3'/C5'), 121.1 (C6''), 118.9 (C3''), 112.8 (C1''), 56.7 (OMe); IR (neat) ν_{max} 3162, 2943, 2845, 1603, 1510, 1335, 1276, 1257, 1108, 1079, 1059, 1028, 852, 801, 757, 753 cm^{-1}. MS (ESI +ve) *m/z* 347 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{19}H_{15}N_4O_3$ 347.1144, found 347.1152 ($[M + H]^+$).

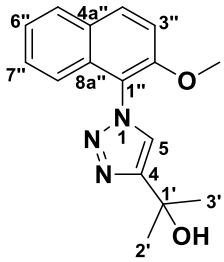
1-(2-Methoxynaphthalen-1-yl)-4-(naphthalen-1-yl)-1*H*-1,2,3-triazole (128)



This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 1-ethynylnaphthalene (0.076 g, 0.5 mmol), $CuSO_4.5H_2O$ (0.012 g, 0.05 mmol) and Na. ascorbate (0.02 g, 0.1 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 12 h to give **128**

(0.08 g, 91%) as a pale yellow waxy solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.3; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58-8.55 (m, 1H, H10'), 8.09 (s, 1H, H5), 8.02 (d, *J* = 10.7 Hz, 1H, H8''), 7.94-7.86 (m, 4H, H5'', H2', H4' and H7'), 7.60-7.49 (m, 4H, H6'', H7'', H9' and H3'), 7.45-7.38 (m, 3H, H4'', H3'' and H8'), 3.93 (s, 3H, OMe); ^{13}C NMR (126 MHz, $CDCl_3$) δ 152.1 (C2''), 146.2 (C4), 134.0 (C1'), 131.9 (C8a''), 131.2 (C4b'), 131.1 (C8b'), 128.9 (C4a''), 128.7 (C4''), 128.5 (C5'), 128.4 (C8'), 128.0 (C4'), 127.9 (C5''), 127.4 (C7'), 126.6 (C7''), 126.5 (C5), 126.0 (C6'), 125.5 (C8''), 125.4 (C3'), 124.7 (C6''), 121.5 (C2'), 119.4 (C3''), 113.0 (C1''), 56.8 (OMe); IR (neat) ν_{max} 3137, 2936, 2846, 1629, 1506, 1462, 1390, 1278, 1264, 1064, 1044, 1021, 905, 803, 778, 749, 663 cm^{-1}. MS (ESI +ve) *m/z* 352 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{23}H_{18}N_3O$ 352.1450, found 352.1459 ($[M + H]^+$).

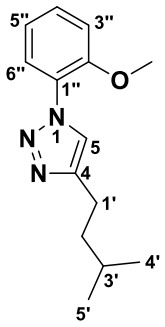
2-(1-(2-Methoxynaphthalen-1-yl)-1*H*-1,2,3-triazol-4-yl) propan-2-ol (129)



This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxynaphthalene **82** (0.05 g, 0.25 mmol) was treated with 2-methylbut-3-yn-2-ol (0.043 g, 0.5 mmol), $CuSO_4.5H_2O$ (0.012 g, 0.05 mmol) and Na. ascorbate (0.02 g, 0.1 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 12 h to give **129** (0.06 g, 86%) as a yellow solid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 40:60 → 100:0). M.P: 122-124 °C; TLC (EtOAc/*n*-hexane 1:4): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, *J* = 9.1 Hz, 1H, H8''), 7.84-7.82 (m, 1H, H5''), 7.69 (s, 1H, H5), 7.44-7.38 (m, 2H, H6'' and H7''), 7.36 (d, *J* = 8.7 Hz, 1H, H4''), 7.14 (dd, *J* = 8.7 Hz, 1H, H3''), 3.87 (s, 3H, OMe), 2.94 (brs, 1H, -OH), 1.77 (s, 6H, H2' and H3'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.0 (C2''), 152.1 (C8a''), 131.8 (C4), 131.1 (C4''), 128.5 (C4a''), 128.3 (C5''), 127.8 (C7''), 124.6 (C8''), 123.0 (C5), 121.3 (C6''),

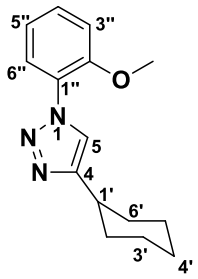
119.4 (C3''), 113.0 (C1''), 68.6 (C1'), 56.6 (OMe), 30.5 (C2' and C3'); IR (neat) ν_{max} 3333, 2981, 2919, 2849, 1632, 1511, 1456, 1359, 1275, 1219, 1143, 1067, 1045, 805, 776, 757 cm^{-1}. MS (ESI +ve) *m/z* 284 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{16}H_{18}N_3O_2$ 284.1399, found 284.1396 ($[M + H]^+$).

4-Isopentyl-1-(2-methoxyphenyl)-1*H*-1,2,3-triazole (130)



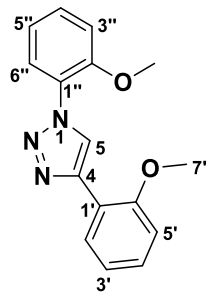
This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxybenzene **118** (0.05 g, 0.33 mmol) was treated with 5-methyl-1-hexyne (0.065 g, 0.66 mmol), $CuSO_4.5H_2O$ (0.017 g, 0.06 mmol) and Na. ascorbate (0.026 g, 0.13 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 12 h to give **130** (0.078 g, 96%) as a pale-yellow oil after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80 $\rightarrow$ 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.83 (s, 1H, H5), 7.73 (dd, *J* = 10.3, 2.2 Hz, 1H, H6''), 7.40-7.35 (m, 1H, H4''), 7.08-7.04 (m, 2H, H5'' and H3''), 3.86 (s, 3H, OMe), 2.82-2.78 (m, 2H, H1'), 1.70-1.60 (m, 3H, H2' and H3'), 0.96 (d, *J* = 7.8 Hz, 6H, H4' and H5'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 151.4 (C2''), 147.9 (C4), 129.8 (C4''), 126.5 (C6''), 125.4 (C5''), 122.7 (C5), 121.1 (C3''), 112.2 (C1''), 55.9 (OMe), 38.4 (C2'), 27.7 (C1'), 23.6 (C3'), 22.4 (C4' and C5'); IR (neat) ν_{max} 2953, 2868, 1601, 1506, 1473, 1285, 1251, 1233, 1122, 1043, 1021, 985, 795, 750 cm^{-1}. MS (ESI +ve) *m/z* 246 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{14}H_{20}N_3O$ 246.1606, found 246.1617 ($[M + H]^+$).

4-Cyclohexyl-1-(2-methoxyphenyl)-1*H*-1,2,3-triazole (131)



This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxybenzene **118** (0.05 g, 0.33 mmol) was treated with ethynyl cyclohexane (0.07 g, 0.66 mmol), $CuSO_4.5H_2O$ (0.017 g, 0.06 mmol) and Na. ascorbate (0.026 g, 0.13 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 12 h to give **131** (0.077 g, 90%) as a pale-yellow oil after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80 $\rightarrow$ 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.5; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (s, 1H, H5), 7.75-7.73 (m, 1H, H6''), 7.40-7.35 (m, 1H, H4''), 7.08-7.04 (m, 2H, H5'' and H3''), 3.86 (s, 3H, OMe), 2.88-2.80 (m, 1H, H1'), 2.16-2.11 (m, 2H, H2' and H6'), 1.85-1.71 (m, 3H, H3', H4' and H5'), 1.51-1.40 (m, 4H, H2', H3', H5' and H6'), 1.34-1.25 (m, 1H, H4'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.0 (C2''), 151.1 (C4), 129.7 (C4''), 126.6 (C6''), 125.5 (C5''), 121.5 (C5), 121.1 (C3''), 112.2 (C1''), 55.9 (OMe), 35.3 (C1'), 33.0 (C2' and C6'), 26.2 (C4'), 26.1 (C3' and C5'); IR (neat) ν_{max} 2929, 2849, 1602, 1504, 1473, 1458, 1282, 1248, 1181, 1124, 1049, 1021, 748 cm^{-1}. MS (ESI +ve) *m/z* 258 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{15}H_{20}N_3O$ 258.1606, found 258.1602 ($[M + H]^+$).

1,4-Bis(2-methoxyphenyl)-1*H*-1,2,3-triazole (132)



This compound was prepared according to the **General procedure IV**, 1-azido-2-methoxybenzene **118** (0.05 g, 0.33 mmol) was treated with 1-ethynyl-2-methoxybenzene (0.09 g, 0.66 mmol), $CuSO_4.5H_2O$ (0.017 g, 0.06 mmol) and Na. ascorbate (0.026 g, 0.13 mmol) in *t*-BuOH:H_2O (1 mL:0.25 mL) at rt for 12 h to give **132** (0.082 g, 88%) as a pale brown liquid after purification by column chromatography over SiO_2 gel (EtOAc/*n*-hexane - 20:80

→ 100:0). TLC (EtOAc/*n*-hexane 1:4): R_f = 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.50 (s, 1H, H5), 8.40 (dd, *J* = 9.6, 2.2 Hz, 1H, H2'), 7.74 (dd, *J* = 9.8, 2.1 Hz, 1H, H6''), 7.41-7.37 (m, 1H, H4''), 7.32-7.28 (m, 1H, H5'') , 7.10-7.04 (m, 3H, H3', H4' and H5'), 6.96 (dd, *J* = 10.4, 1.0 Hz, 1H, H3''), 3.90 (s, 3H, OMe), 3.84 (s, 3H, H7'); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.7 (C6'), 151.6 (C2''), 142.6 (C4), 130.0 (C2'), 128.8 (C4''), 127.7 (C4'), 126.5 (C6''), 125.7 (C3'), 125.2 (C5), 121.1 (C5''), 121.0 (C1'), 119.5 (C3''), 112.3 (C1''), 110.9 (C5'), 55.9 (OMe), 55.4 (C7'); IR (neat) ν_{max} 2936, 2838, 1601, 1506, 1489, 1465, 1287, 1247, 1161, 1130, 1078, 1022, 748, 689 cm^{-1}. MS (ESI +ve) *m/z* 282 ($[M + H]^+$, 100%); HRMS (ESI +ve TOF) calcd for $C_{16}H_{16}N_3O_2$ 282.1243, found 282.1234 ($[M + H]^+$).

Synthesis of Azides and alkynes:

The known azides **82**[82], **97**[83] and **99**[84] were prepared from their corresponding 1-naphthylamines according to the method of Hu *et al.*[85] All alkynes were commercially sourced expect for 1-ethynylnaphthalene,[86] 1-ethynyl-4-nitrobenzene,[87] and 2-ethynyl-1,3,5-trimethylbenzene[88] which were prepared by literature methods.

6.5 – Experimental procedures for biological and pharmacological assays

6.5.1 – Minimum inhibitory concentration (MIC)

6.5.1.1 – Primary screening

The primary screening experiments were performed at University of Western Australia by Dr. Katherine Hammer and Dr. Daniel Knight. MIC analyses were executed on *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *Staphylococcus aureus* (NCTC 10442), *Enterococcus faecalis* (ATCC 29212) and *Escherichia coli* (ATCC 25922) in Mueller Hinton (MH) broth and incubation was completed in ambient air at 35 °C for 24 h.

Streptococcus pneumoniae (ATCC 49619) was cultivated in MH broth with 2.5% lysed horse blood and incubated with 5% CO_2 at 35 °C for 24 h. MIC tests for *Clostridium difficile* (ATCC 700057) and *Clostridium difficile* (NSW132 – RT027) were performed in Brucella broth supplemented with haemin and vitamin K and incubation was completed anaerobically at 35 °C for 48 h. Every compound was dissolved in DMSO at 3 – 5 mg/mL and then diluted to 512 μg/mL with sterile, distilled water. The compounds were then diluted sequentially in 100 μL volumes of sterile, distilled water in a 96-well microtitre plate. Every testing bacterium in double strength broth (100 μL) was then added to each well and incubated as explained above. Testing concentrations of every compound ranged from 0.25 μg/mL to 128 μg/mL. Vancomycin and a control well (i.e. no antibacterial compound present) were involved in the assays. A DMSO control (5% v/v) was also analyzed to confirm the solvent did not inhibit bacterial growth. The test was performed in triplicate for each organism and compound mixture and the average MIC values were verified. The lowest concentration that inhibits bacterial development completely will be recorded as the compound's MIC value. The MBC (minimum bactericidal concentration) was also noted as the lowest concentration that caused cellular death.

6.5.1.2 – Secondary screening and cytotoxicity assays (CO-ADD)

Antibacterial compounds were screened at CO-ADD for antimicrobial testing by complete cell development inhibition analyses. The inhibition of development was measured against five bacterial strains: *Escherichia coli (*ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 43300), and two fungi: *Candida albicans* (ATCC 90028) and *Cryptococcus neoformans* (ATCC 208821). After MIC screening, the

compounds were tested for cytotoxicity against a human embryonic kidney cell line (HEK293) by ascertaining their CC_{50} value. Antibacterial compound solution was prepared in DMSO to a final screening concentration of 32 μg/mL and serially diluted 1:2 fold for 8 times. Each compound concentration was prepared in 384 – well plates, non-binding surface (NBS) plate (Corning 3640) for each bacterial/fungal strain and tissue-culture treated (Corning 3712/3764) black for mammalian cell types, all in duplicate (n = 2) and keeping the final DMSO concentration to a maximum of 0.5%. All the compound's preparation was accomplished using liquid handling robots.

6.5.1.3 – Bacterial Inhibition

All of the bacterial strains were cultured in cation-adjusted MH broth at 37 °C overnight. Each compound culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1 – 3.5 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD600), then added to each well of the compound-containing plates, giving a cell density of 5×10^5 CFU/mL and a total volume of 50 μL. All plates were protected and incubated at 37 °C for 18 h without shaking. Inhibition of bacterial development was verified by calculating absorbance at 600 nm (OD600), using a Tecan M1000 Pro monochromator plate reader. The percentage of growing inhibition was determined for each well, utilizing the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references. The MIC was revealed as the lowest concentration at which bacterial development was completely inhibited, identified by an inhibition ≥ 80%. Vancomycin was utilized as a positive control for Gram-positive bacteria and colistin was used as a positive control for Gram-negative bacteria. Each antibiotic control was given in four concentrations,

with two above and two below its MIC value, and plated into the first eight wells of column 23 of the 384 – well NBS plates.

6.5.1.4 – Fungal Inhibition

Fungal strains were cultured for three days on Yeast Extract-Peptone Dextrose agar at 30 °C. A yeast suspension of 1×10^6 to 5×10^6 CFU/mL (as determined by OD530) was prepared from five colonies. The suspension was diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5×10^3 CFU/mL and a total volume of 50 µL. All plates were protected and incubated at 35 °C for 36 h without shaking. The inhibition of *C. albicans* development was decided by evaluating absorbance at 630 nm (OD630), and the inhibition of *C. neoformans* development was decided by assessing the difference in absorbance between 600 and 570 nm (OD600 – 570), after the addition of resazurin (0.001 % final concentration) and incubation at 35 °C for 2 h. The absorbance was measured using a Biotek Multiflo Synergy HTX plate reader. The percentage of growing inhibition for both fungi were calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate.

The MIC was decided as the lowest concentration at which the fungal development was fully inhibited, defined as an inhibition ≥ 80% for *C. albicans* and an inhibition ≥ 70% for *C. neoformans*. Due to the higher difference in growth and inhibition, a lower threshold was employed to the data for *C. neoformans*. Fluconazole was used as a positive control for anti-fungal assay of *C. albicans* and *C. neoformans*. The antifungal control was given in four concentrations, with two above and two below its MIC value, and plated into the first eight wells of column 23 of the 384 – well NBS plates.

6.5.2 – Cytotoxicity Assay

HEK293 cells were counted manually in a Neubauer haemocytometer and then plated in the 384 – well plates containing the compounds to give a density of 5000 cells/well in a final volume of 50 μL. Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) was used as a growth media and the cells were incubated together with the compounds for 20 h at 37 °C in 5% CO_2. Cytotoxicity (cell viability) was evaluated by fluorescence, ex: 560/10 nm, em: 590/10 nm (F560/590), after addition of 5 μL of 25 μg/mL resazurin (2.3 μg/mL final concentration) and after incubation for further 3 h at 37 °C in 5% CO_2. The intensity of fluorescence was measured using a Tecan M1000 Pro monochromator plate reader, using automatic gain calculation. CC_{50} (concentration at 50% cytotoxicity) values were calculated by curve-fitting the inhibition values vs. log(concentration) using a sigmoidal dose-response function, with variable fitting values for bottom, top and slope. Tamoxifen was used as a positive cytotoxicity control, used in eight concentrations in two-fold serial dilutions with 50 μg/mL as the highest concentration tested.

6.5.3 – *In vivo* CDI mouse model (Monash University)

6.5.3.1 – Study design

The *in vivo* CDI mouse model study was performed by Prof. Dena Lyras, Dr. Melanie Hutton and Dr. Amy King. Mice were pre-treated with an antibiotic cocktail containing kanamycin (0.4 mg/mL; Amresco), gentamicin (0.035 mg/mL; Sigma), colistin (850 mg/mL; Sigma), metronidazole (0.215 mg/mL; Sigma), vancomycin (0.045 mg/mL; Sigma) and cefaclor (0.3 mg/mL; Sigma) in the drinking water for seven days, followed by three days of cefaclor alone to obstruct the commensal microbiota to bring infection with *C. difficile*. The mice were shifted to clean cages containing plain drinking water on the day of

infection. Group of infected five mice with 10^5 *C. difficile* spores of the RT027 strain M7404. After six hours post-infection, 2.5 mg of the selected compounds (in 10% aqueous DMSO) were given to the mice *via* oral gavage. The mice were then administered fresh compound every 12 h after the initial dose. The mice were monitored twice in a day for weight loss and other physiological signs of infection. The blood was collected and left to clot at room temperature prior to centrifugation. The serum and faeces were collected and frozen at – 80 °C for later pharmacokinetic evaluation. Note that mice were euthanized according to animal ethics guidelines: i.e. if the mice lose ≥ 10 % of their total body weight in the first 24 h or ≥ 15 % at any point after 24 h.

6.5.3.2 – Sample preparation

Each compound was dissolved in 100% DMSO to attain a stock concentration of 250 mg/mL. Twenty minutes before the administration, the compound stock solution was diluted (1:10) with warm water (37 °C) to accomplish a final concentration of 25 mg/mL. The compounds were vortexed and carried to the animal house floating in warm water in an attempt to maintain a homogeneous solution. The compounds were then vortexed again immediately preceding to gavaging the mice. Each mouse received 100 μL of solution containing 2.5 mg of compound – this equates to a dose of 100 mg/kg (based on an average 25 g mouse).

6.5.4 – Comparative solubility assay

6.5.4.1 –Procedure

Five milligrams of the target compound was fully dissolved in 50 μL DMSO. Small aliquots (5 μL) of H_2O were added to the DMSO solution with manual agitation after each

addition; additions were continued until a visible turbidity or cloudiness was apparent that did not fade after mixing. The quantity of H_2O needed for precipitation of the compound was recorded; this value was then divided by the standard value (i.e. 15 μL = quantity of H_2O required to precipitate lead compound **AVX-13616**) to obtain a comparative solubility ratio. This ratio aided to assess the observed change in solubility that was observed for various compounds.

7.0 – References

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